

Molecular epidemiology and phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field isolates in Korea

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Abstract Porcine epidemic diarrhea virus (PEDV) has caused devastating enteric disease in Korean pig farms since its first identification in 1992 in Korea. In the present study, the molecular epidemiology, genetic diversity, and phylogenetic relationship of Korean PEDV field isolates to other reference strains were analyzed using the complete E gene. Genetic analysis showed that each PEDV group had several unique characteristics, which indicated that a specific group PEDVs may be differentiated from another group PEDVs. Phylogenetic analysis showed that recent prevalent Korean PEDV field isolates are closely related to the Chinese field strains and differ genetically from the European strains and the vaccine strains used in Korea, which raises questions of whether a new-type PEDV vaccine may be necessary for preventing PEDV infection more effectively in Korea. Notably, a large deletion identified only in the attenuated DR13 can be utilized as a genetic

marker, and the methods developed in this study will help to rapidly detect and differentiate PEDVs.

Introduction

Porcine epidemic diarrhea virus (PEDV) is an enveloped, single-stranded RNA virus belonging to the family *Coronaviridae*, subfamily *Coronavirinae*, genus *Alphacoronavirus*. PEDV was first reported in Belgium and the United Kingdom in 1978 [22]. Since the first identification of PEDV, outbreaks of PEDV have been reported in many swine-producing countries, notably in Europe and Asia [23]. Porcine epidemic diarrhea (PED), caused by PEDV, is an acute, highly contagious, and devastating enteric disease that is characterized by severe diarrhea, dehydration and a high mortality rate in swine, resulting in severe economic losses in the European and Asian swine industry [23].

Coronaviruses (CoVs) have a genome organization with a common set of five genes arranged in a conserved order [5]. The polymerase gene, covering the 5' 70 % of the genome, encodes the replicase polyproteins. The genes for the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) are located downstream of the polymerase gene [5]. A variety of genes, which are called accessory genes and encode accessory proteins whose sequence and number differ among the different species of CoV, are studied between the structural genes [15].

One of the four CoV structural proteins, the E protein, is a small transmembrane protein of 76–109 amino acids in length [3, 25]. The amino acid sequence of the E protein is quite divergent among different CoVs notwithstanding that its predicted structure is highly conserved [31]. The CoV E protein has a pivotal role in the assembly of virions, where it may induce membrane curvature or aid in membrane

GenBank accession numbers of all sequences described in this study are indicated in the figure legends.

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scission [3, 10, 11, 25]. Beyond assembly, the E protein has functions during infection, including in virus egress and in the host stress response [2, 4, 8, 17, 18, 26, 34]. In addition, the E protein has ion channel activity and interacts with host proteins [1, 21, 24, 29, 31–33]. In the case of severe acute respiratory syndrome (SARS)-CoV, the deletion of the E protein modestly reduces its growth *in vitro* and *in vivo* [6, 7], resulting in an attenuated virus. In other CoVs, deletion of the E protein results in either the complete absence of infectious virus or a severe reduction in virus titer [12, 16, 18]. However, unlike other CoVs, in the case of PEDV, little is known about the functions of the E protein.

In Korea, PEDV was first isolated in 1992 [13]. Since then, it has been frequently detected in many provinces and has become one of the most important viral enteric diseases. In spite of using the vaccine strategy at present, damage caused by PEDV infection is continuous and serious in Korea. To better control and prevent PEDV infection, it is necessary to investigate the molecular epidemiology of PEDV field isolates in Korea. In this study, therefore, we investigate the molecular epidemiology and genetic diversity of the Korean PEDV field isolates and analyze the phylogenetic relationship of the Korean PEDV field isolates to other previously reported PEDV reference strains. In addition, we develop methods that can differentiate the attenuated DR13 strain, which has been used for manufacture of the most commonly used live oral PED vaccine in Korea, from all the other PEDVs. The present study focused on the E gene for analysis because of the characteristics described above.

Materials and methods

Korean PEDV field isolates

Porcine fecal and intestinal samples were collected between May 2010 and May 2011 from eight swine farms in four provinces of Korea. All of the pigs showed signs of watery diarrhea and dehydration at the time of sample collection. Fresh samples were collected from individual pigs, placed into a sterile specimen container, and submitted to the Department of Veterinary Medicine Virology Laboratory, College of Veterinary Medicine, Seoul National University. These intestinal and fecal samples had been confirmed positive for PEDV by reverse transcription polymerase chain reaction (RT-PCR) [19].

PEDV vaccine strains used in Korea

To minimize losses caused by PEDV infection, live vaccines (attenuated DR13, KPED-9 and P-5V strains) and an inactivated vaccine (SM98-1 strain) have been used in

Korea. Therefore, these vaccine strains were included in the present study for comparative analysis with Korean PEDV field isolates. The attenuated DR13 strain was derived from the virulent DR13 strain by serial propagation in Vero cells [27], and it was used for manufacture of the Korean live oral PED vaccine by Green Cross Veterinary Product Co., Ltd. (Yongin, Korea). The attenuated DR13 strain was kindly provided by Green Cross Veterinary Product Co., Ltd. KPED-9 and P-5V strains, used for manufacture of the Korean and Japanese live PED vaccines, were kindly supplied by Green Cross Veterinary Product Co., Ltd., and the Nisseiken regional distributor in Korea, respectively. Virulent SM98-1 strain used for manufacture of the Korean inactivated PED vaccine was also provided by Green Cross Veterinary Product Co., Ltd.

RNA extraction and reverse transcription (RT)

PEDV-positive fecal and intestinal samples were prepared as 10 % (v/v) suspensions with phosphate-buffered saline (PBS; 0.1 M, pH 7.2). The prepared sample suspensions were vortexed and centrifuged for 10 min at $4800 \times g$. RNA was extracted from a 250- μ l starting volume of the centrifuged 10 % sample suspensions using TRIzol LS (Invitrogen Corp.) according to the manufacturer's instructions. Viral RNAs of the vaccine strains (attenuated DR13, KPED-9, P-5V and SM98-1) were also extracted in the same manner as described above. Reverse transcription (RT) was carried out with random hexamer primers (TaKaRa Bio Inc.), and the cDNA was immediately used for amplification or stored at -20°C .

PCR amplification of the E gene

Primers targeting the ORF3 and M genes (corresponding to nt 25,323 to 25,881 of CV777) were designed for generating the full-length E gene of PEDV. The primers were PEDEF (forward), 5'-GCTGACCTACATCTGTTGCG-3', and PEDER (reverse), 5'-GCGTCAAAAAGTGACAGTGC-3'. The size of the amplified product was predicted to be 559 bp. PCR was performed with a commercial amplification system (Perkin-Elmer, Applied Biosystems) as described previously [19] under the following conditions: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52.9°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min.

Cloning of cDNA and sequencing

The RT-PCR products for the complete E gene were analyzed by 1.5 % agarose gel electrophoresis and visualized by ultraviolet illumination after ethidium bromide staining. Bands of the correct size were excised and purified using a QIAquick Gel Extraction Kit (QIAGEN) according to the

manufacturer's instructions. The purified RT-PCR products corresponding to the full-length E gene of PEDV were cloned into the pDrive cloning vector (QIAGEN) as described previously [19], and the cloned plasmids were purified using a QIAprep[®] Spin Miniprep Kit (QIAGEN) before sequencing. Sequencing of plasmid DNA was carried out at least twice in both directions at the Genotech Institute (Genotech Co., Ltd.) using T7 and SP6 primers and an automated DNA sequencer (ABI system 3700; Applied Biosystems Inc.).

Molecular analysis

The nucleotide sequences of the full-length E genes of PEDVs were aligned using ClustalX version 1.83 [30], edited using Bioedit version 7.0.5 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>), and compared with those of PEDV reference strains in the GenBank database. Sequence similarity analysis was performed for the aligned nucleotide and amino acid sequences using MegAlign software (DNAStar Inc., Madison, WI, USA). Phylogenetic analysis based on the nucleotide alignments was conducted by using the neighbor-joining method and the minimum-evolution method of Molecular Evolutionary Genetics Analysis (MEGA version 5.05) with a pairwise distance [28]. The Korean PEDV field isolates, PEDV vaccine strains, and the other PEDV reference strains used for sequence alignment, sequence analysis and phylogenetic analysis are indicated in the figure legends.

Semi-nested RT-PCR for the differentiation of the attenuated DR13 strain from all the other PEDVs

Semi-nested RT-PCR primers were designed to discriminate the attenuated DR13 strain from all the other PEDVs based on the sequence alignment results of the complete E genes of the Korean PEDV field isolates, PEDV vaccine strains, and PEDV reference strains described above. The forward primer (PEDENF, 5'-TTCGTA CTCTTTTCTGCTTA T-3') was a newly designed gene-specific primer, and the reverse primer (PEDER) was identical to that utilized in the first-round PCR reaction. Semi-nested PCR was carried out using the first-round PCR products, diluted at least 100-fold, under the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 50.7 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 7 min.

Results

Phylogenetic analysis of the E gene

Phylogenetic analysis based on the complete E gene fragments of the Korean PEDV field isolates, PEDV vaccine

strains, and PEDV reference strains confirmed that all PEDVs, including the Korean field isolates, fell into three groups (Fig. 1). One group comprised the virulent DR13 strain, eight Korean field isolates, and five Chinese strains. The second group consisted of the live vaccine strains, attenuated DR13, KPED-9 and P-5V. The third group included the CV777, Chinju99, virulent SM98-1 strain of the Korean inactivated PED vaccine, and four Chinese strains.

Genetic analysis of the E gene

Sequences of the complete E genes of the Korean PEDV field isolates and PEDV vaccine strains were determined and compared with the PEDV reference strains. All Korean PEDV field isolates and PEDV vaccine strains except the attenuated DR13 strain had a single ORF of 231 nucleotides encoding a protein of 76 amino acids with a predicted *Mr* of 8.8-8.9 kDa. On the other hand, the attenuated DR13 strain had a single ORF of 210 nucleotides encoding a protein of 69 amino acids with a predicted *Mr* of 8.0 kDa because of a 21-nucleotide deletion at position 67-87. All Korean PEDV field isolates and PEDV vaccine strains, including the attenuated DR13 strain, had a conserved sequence (CTAGAC) at 10 nucleotides upstream from the initiator ATG, as previously recognized in Br1/87 [9].

Sequence analysis of the complete E genes showed that all PEDVs, including the Korean field isolates, fell into three groups as can be seen in the phylogenetic tree (Fig. 1). Each group had unique differences in its sequence. Group 1 had two specific nucleotide changes (C165T and G194A) that were not found in other groups. The latter of these two changes led to an amino acid change (R65Q). Group 2 showed two specific nucleotide changes (T32C and T42C), and the former resulted in an amino acid change (V11A). Group 3 exhibited one specific nucleotide change (T166C) (Fig. 2). These results indicated that these specific nucleotide differences observed in each PEDV group may be used to differentiate PEDVs of a specific group from those of another group, although more PEDVs need to be analyzed. In addition, a large deletion identified only in the attenuated DR13 strain, which has been used for manufacture of the most commonly used live oral PED vaccine in Korea, can be utilized as a genetic marker.

Comparison of E gene sequences

The sequence identity results based on the complete E genes of all PEDVs including the Korean PEDV field isolates are shown in Table 1, and these revealed that group 1 PEDVs have 96.5-100 % (97.3-100 %) nucleotide (deduced amino acid) sequence identity to each other, and they have 95.6-97.6 % (94.2-97.3 %) and 93.9-98.2 %

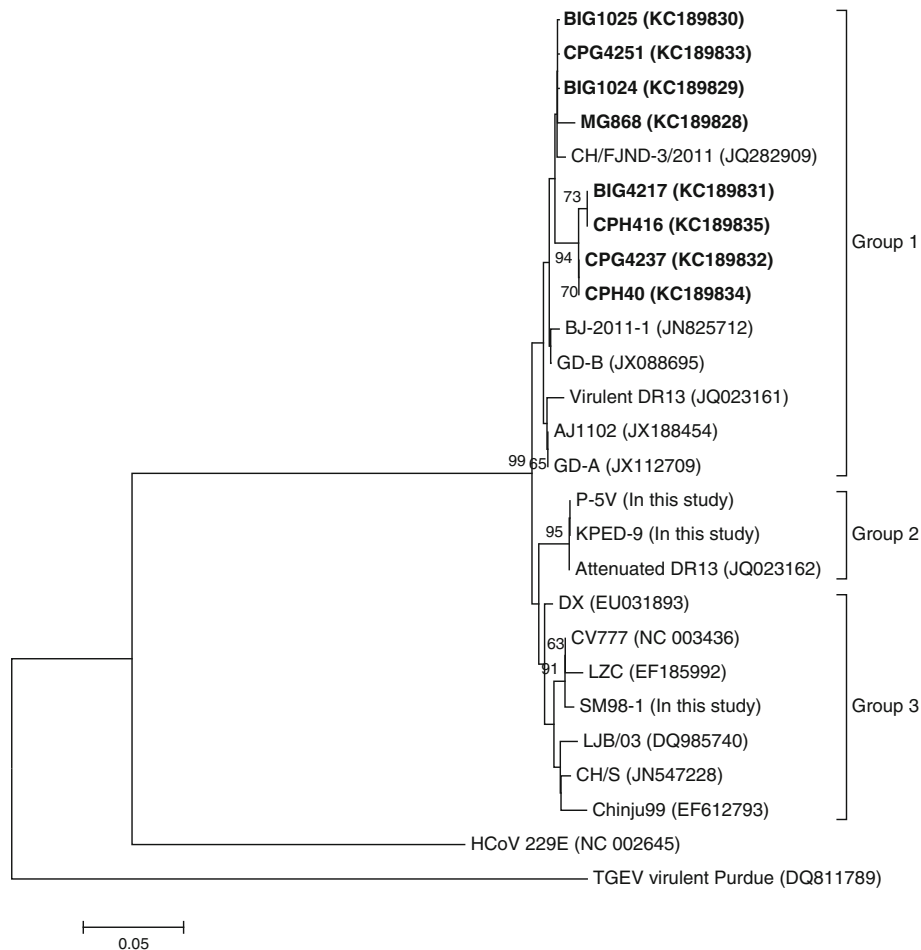


Fig. 1 Relationships between Korean PEDV field isolates (accession nos. KC189828-KC189835 in **boldface**) and other PEDV reference strains based on the full-length E gene. Phylogenetic trees were constructed using the neighbor-joining method in MEGA version 5.05 with pairwise distances [28]. Bootstrap values (based on 1000 replicates) for each node are given if >60 %. The *scale bar* indicates nucleotide substitutions per site. Accession numbers for the Korean PEDV field isolates and the other PEDV reference strains used in the analysis are follows: Virulent DR13 (JQ023161), MG868

(KC189828), BIG1024 (KC189829), BIG1025 (KC189830), BIG 4217 (KC189831), CPG4237 (KC189832), CPG4251 (KC189833), CPH40 (KC189834), CPH416 (KC189835), BJ-2011-1 (JN825712), CH/FJND-3/2011 (JQ282909), AJ1102 (JX188454), GD-A (JX112709), GD-B (JX088695), attenuated DR13 (JQ023162), KPED-9 (this study), P-5V (this study), CV777 (NC_003436), Chinju99 (EF612793), SM98-1 (this study), LZC (EF185992), DX (EU031893), CH/S (JN547228), LJB/03 (DQ985740)

(94.7-98.6 %) sequence identity to group 2 and 3, respectively. Group 2 PEDVs have 100 % (100 %) sequence identity to each other, and they have 96.1-97.8 % (94.2-97.3 %) sequence identity to the members of group 3. Group 3 PEDVs have 96.9-99.5 % (96.0-100 %) sequence identities to each other.

Differentiation of the attenuated DR13 strain from all the other PEDVs by semi-nested RT-PCR using a large deleted region

All PEDVs, including eight Korean PEDV field isolates and PEDV vaccine strains, were detected by RT-PCR using a pair of primers (PEDEF and PEDER) (Fig. 3a). Subsequently, the attenuated DR13 strain was rapidly differentiated from all the

Fig. 2 Comparison of the nucleotide (a) and deduced amino acid (b) sequences of the full-length E genes of Korean PEDV field isolates and other PEDV reference strains. Asterisks represent nucleotides (a) and amino acids (b) that are identical to those of the virulent DR13 strain. Dashed lines represent missing nucleotides (a) and amino acids (b). The start codon (ATG) and stop codon (TGA) are underlined. Regions corresponding to the primers are underlined and labeled above the sequence as PEDEF, PEDENF and PEDER

other PEDVs by semi-nested RT-PCR with PEDENF and PEDER primers. Using these primers, cDNA was amplified from all of the PEDVs except the attenuated DR13 strain, which could not be amplified because of the large deletion in its E gene (Fig. 3b). This assay can therefore be used to discriminate the attenuated DR13 strain from all of the other PEDVs.

(a)

-121 PEDEF -102 -10 -1
GCTGACCTACATCTGTTGGC CTAGACGAGT

	1	20 21	40 41	60 61	PEDEF	80 81	100 101	120				
Virulent DR13	ATGCTACAAT	TAGTGAATGA	TAATGGTCTA	GTAGTTAATG	TTATACTTTG	GCTTTTCGTA	CCTTTTTTC	TGCTTATTAT	AAGCATTACT	TTGCTCCAAT	TGGTTAATCT	GTGCTTCACT
MG868	*****	*****	*****C**	*****	*****	*****CA	*****	*****	*****	*****	*****G**	*****
BIG1024	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
BIG1025	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
BIG4217	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
CPG4237	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
CPG4251	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
CPH40	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
CPH416	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
BJ-2011-1	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
CH/FJND-3/2011	*****	*****	*****	*****	*****T**	*****C*	*****	*****	*****	*****	*****	*****
AJ1102	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
GD-A	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
GD-B	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****	*****	*****
Attenuated DR13	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
KPED-9	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
P-5V	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
CV777	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****C	*****	*****
Chinju99	*****	*****	*****	*****	*****	*****T**	*****	*****	*****	*****C	*****	*****T**
SM98-1	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****C	*****	*****T**
LZC	*****	*****	*****	*****	*****	*****C*	*****	*****	*****	*****C	*****	*****
DX	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
CH/S	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****
LJB/03	*****	*****	*****	*****	*****	*****C*T**	*****	*****	*****	*****C	*****	*****

	121	140 141	160 161	180 181	200 201	220 221	231					
Virulent DR13	TGTCACCGGT	TGTGTAATAG	CGCAGTTTAC	ACACCTATAG	GGCGTTTGTA	TAGAGTTTAT	AAGTCTTACA	TGCAAAITGA	COCCCTCCOC	AGCACTGTTA	TTGACGTAIA	A
MG868	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
BIG1024	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
BIG1025	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
BIG4217	*****	*****C**	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****C**A**
CPG4237	*****	*****C**	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****A**
CPG4251	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
CPH40	*****	*****C**	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
CPH416	*****	*****C**	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****C**A**
BJ-2011-1	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
CH/FJND-3/2011	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
AJ1102	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
GD-A	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
GD-B	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
Attenuated DR13	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
KPED-9	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
P-5V	*****	*****	*****	*****	*****	*****	*****	*****A**	*****T	*****T	*****	*****
CV777	*****	*****	*****T	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
Chinju99	*****	*****	*****T	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
SM98-1	*****	*****	*****T	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
LZC	*****	*****	*****T	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
DX	*****	*****	*****	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
CH/S	*****	*****	*****	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****
LJB/03	*****	*****	*****	*****	*****OC**	*****	*****	*****A**	*****T	*****T	*****	*****

419 PEDEF 438
..... GCACTGTCACCTTTTGACGC

(b)

	1	20 21	40 41	60 61	76			
Virulent DR13	MLQLVNDNGL	VNVNVLNLFV	LFLLIISIT	FVQLVNLCT	CHRLNSAVY	TPIGRLYRVY	KSYMIDPLP	STVIDW
MG868	*****	*****	*****S**	*****	*****	*****	*****	*****
BIG1024	*****	*****	*****	*****	*****	*****	*****	*****
BIG1025	*****	*****	*****	*****	*****	*****	*****	*****
BIG4217	*****	*****	*****	*****	*****	*****	*****	*****
CPG4237	*****	*****	*****	*****	*****	*****	*****	*****
CPG4251	*****	*****	*****	*****	*****	*****	*****	*****
CPH40	*****	*****	*****	*****	*****	*****	*****	*****
CPH416	*****	*****	*****	*****	*****	*****	*****	*****
BJ-2011-1	*****	*****	*****	*****	*****	*****	*****	*****
CH/FJND-3/2011	*****	*****	*****	*****	*****	*****	*****	*****
AJ1102	*****	*****	*****	*****	*****	*****	*****	*****
GD-A	*****	*****	*****	*****	*****	*****	*****	*****
GD-B	*****	*****	*****	*****	*****	*****	*****	*****
Attenuated DR13	*****	A*****	*****	*****	*****	*****R**	*****	*****
KPED-9	*****	A*****	*****	*****	*****	*****R**	*****	*****
P-5V	*****	A*****	*****	*****	*****	*****R**	*****	*****
CV777	*****	*****	*****	*****	*****	*****R**	*****	*****
Chinju99	*****	*****	*****	*****	*****	*****R**	*****	*****
SM98-1	*****	*****	*****	*****	*****	*****R**	*****	*****
LZC	*****	*****	*****	*****	*****	*****R*ER*	*****	*****
DX	*****	*****	*****	*****	*****	*****R**	*****	*****
CH/S	*****	*****	*****	*****	*****	*****R**	*****	*****
LJB/03	*****	*****L*	*****	*****	*****	*****R**	*****	*****

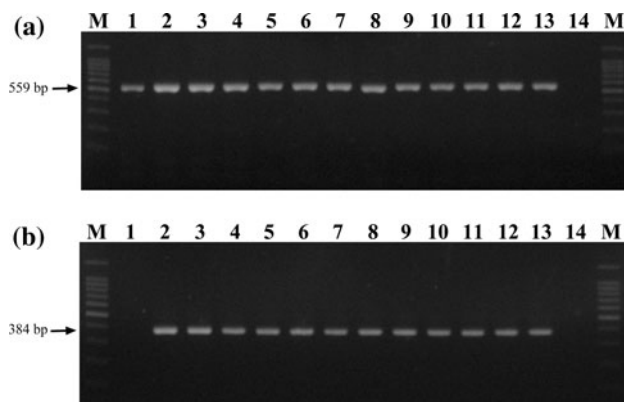


Fig. 3 RT-PCR (a) using the PEDEF/PEDER primer pair and semi-nested RT-PCR (b) using the PEDENF/PEDER primers with the Korean PEDV field isolates and PEDV vaccine strains used in Korea. From left to right: lane M 100-bp DNA ladder; lane 1, Attenuated DR13 strain of the Korean live oral PED vaccine; lane 2, KPED-9 strain of the Korean live PED vaccine; lane 3, P-5V strain of the Japanese live PED vaccine; lane 4, SM98-1 strain of the Korean inactivated PED vaccine; lane 5, Virulent DR13 strain; lane 6, MG868; lane 7, BIG1024; lane 8, BIG1025; lane 9, BIG4217; lane 10, CPG4237; lane 11, CPG4251; lane 12, CPH40; lane 13, CPH416; lane 14, negative control

Discussion

In this study, the complete E genes of the Korean PEDV field isolates were amplified by RT-PCR, cloned, and sequenced to investigate their molecular epidemiological characteristics and their genetic diversity. The phylogenetic relationship between the Korean PEDV field isolates and other previously reported PEDV reference strains was also analyzed. Moreover, methods that can rapidly detect and differentiate the live PEDV vaccine strains used in Korea, notably the attenuated DR13 strain, from all the other PEDVs were developed.

All Korean PEDV field isolates and PEDV vaccine strains (excluding the attenuated DR13 strain) have only point mutations in the E gene, and all PEDVs including the attenuated DR13 have CTAGAC 10 nucleotides upstream of the initiator ATG of the E gene, as previously recognized in Br1/87 [9]. These sequences are hexameric motifs common to CoVs and are similar to the hexameric motifs XUA(A/G)AC found adjacent to other PEDV ORFs. These hexameric motifs have been proposed to be the start sites for the transcription of subgenomic mRNAs [14]. The attenuated DR13 strain has a 21-nucleotide deletion at position 67-87 of its E gene, resulting in a deletion mutant of 69 amino acids. The deletion mutant in the E protein of PEDV was first identified in the present study by comparative analysis of the full-length E genes of PEDVs.

Genetic analysis based on the complete E gene showed that each PEDV group had several unique characteristics,

and these results indicated that specific groups of PEDVs such as the live vaccine strains used in Korea may be differentiated from other groups of PEDVs based on specific nucleotide sequence differences, but more PEDVs need to be analyzed for more accurate analysis. In particular, sequencing the E gene can be used for discrimination between the attenuated DR13 strain and all of the other PEDVs including KPED-9, P-5V, and wild-type PEDVs because of large deletion that has only been identified in the attenuated DR13 strain, which is used for manufacture of the most common live oral PED vaccine in Korea.

Genetic and phylogenetic analysis using the complete E gene showed that recent prevalent Korean PEDV field isolates are closely related to Chinese field strains and differ genetically from the vaccine strains (attenuated DR13, KPED-9, P-5V, and SM98-1) used in Korea as well as European strains, indicating that there may be a new prevailing PEDV genotype in Korea. These results are somewhat similar to those obtained in our previous study using the full-length M and ORF3 genes [20]. However, studies on the E gene of PEDV have almost never been reported although the E gene plays an important role in the biology of CoV, as described above, and to our knowledge, this is the first report of a molecular epidemiological characterization of PEDVs using the E gene. Moreover, this study shows that although the E gene is smaller than the other structural genes, it is sufficient to produce meaningful insights into the epidemiology of PEDV in Korea, and additional studies on the E gene should increase our understanding of the genetic relationships among PEDVs from a wider range of countries, their diversity, and the global epidemic situation of PEDV.

To minimize losses caused by PEDV infection, two strategies are suggested: The first is to protect against PEDV infection in advance by immunization with the PED vaccine. As the damage caused by PED has showed steady increase, PEDV vaccines have been developed and are commonly used in Korea. However, in spite of the use of the vaccine, damage caused by PED outbreaks is continuous and serious in Korean pig farms. Recent prevalent Korean PEDV field isolates analyzed in this study differ from members of the group including the vaccine strains, and therefore, this result raises questions about whether a new-type PEDV vaccine may be necessary for more effective prevention of PEDV infection. The second strategy is to prevent the spread of PEDV more effectively by rapid detection of PEDV when PED occurs. The methods developed in this study can not only detect PEDV rapidly but also differentiate the live PEDV vaccine strains used in Korea, notably the attenuated DR13 strain of the most commonly used live oral PED vaccine in Korea, from all the other PEDVs.

The present study allows a better understanding of the molecular epidemiology, genetic diversity, and phylogenetic relationships of Korean PEDV field isolates with the other PEDV reference strains. Moreover, the methods developed in this study can be utilized as a useful tool for the rapid detection and differentiation of PEDVs.

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