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Short communication

Comparative genome analysis and molecular epidemiology of the reemerging porcine epidemic diarrhea virus strains isolated in Korea

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV), a member of the *Coronaviridae* family, is an enveloped, positive-sense, single-stranded RNA virus, which causes severe diarrhea and dehydration in suckling pigs. We detected three PEDV strains from ten small intestine samples from piglets with acute diarrhea and we determined the complete genome sequences of the reemerging Korean PEDV field isolates, except for the noncoding regions from both ends. The complete genome sequences of the strains were identical or almost identical (one synonymous single-nucleotide polymorphism (SNP) in the ORF1a/1b genomic sequence). Interestingly, comparative genome analysis of recent Korean PEDV isolates and other strains revealed that the complete genome sequences of recent Korean strains were almost identical (99.9%) to those of the US PEDV strains isolated in 2013. These results suggest that the three reemerging Korean strains are distinct from previous endemic Korean PEDV strains and has been recently introduced into Korea from oversea with high likelihood.

1. Introduction

Porcine epidemic diarrhea virus (PEDV) is a member of the family *Coronaviridae*, subfamily *Coronavirinae*, and genus *Alphacoronavirus*, which include some human and bat coronaviruses. PEDV containing a positive-sense, single-stranded RNA genome, causes severe diarrhea and dehydration in suckling piglets (Song and Park, 2012). Since the first report of isolation in Europe in 1978 (Pensaert and de Bouck, 1978), PEDV has become an economic concern in the swine industry in Europe and Asia (Song and Park, 2012).

In late 2010, various Chinese strains of PEDV that were clinically more severe than the classical strains, with 80–100% morbidity and 50–90% mortality in suckling piglets, were detected (Li et al., 2012). In April 2013, PEDV outbreaks were confirmed in the US for the first time and the isolates showed very close relationship with the Chinese isolate AH2012. A previous study showed that the emergent US PEDV strains were likely introduced into the US through intercontinental transmission from China (Huang et al., 2013).

In Korea, PEDV was first isolated in 1992, followed by a large two-year-long outbreak. Despite the use of vaccines, frequent occurrence of PEDV was detected across the country, mainly during the winter season (Chae et al., 2000; Kweon et al., 1993). Since late November 2013, PEDV has reemerged in Korea and caused significant economic losses in the swine industry. This study aimed to determine the complete genome sequence of the reemerging Korean PEDV strain and to investigate their genetic relationship with other strains using comparative genome analysis and phylogenetic analysis.

2. Materials and methods

Ten small intestine samples were collected from dead piglets from two commercial pig farms in Korea. The piglets died following acute watery diarrhea. The macroscopic features of the intestines were typical of PEDV infections, including yellowish contents and distended appearance. To detect PEDV genome, M gene-targeted RT-PCR was performed (Kim et al., 2000) using total RNA from mucosal scrapings. Three of ten samples were positive in the PEDV specific RT-PCR. To investigate the origin of the reemerging Korean PEDV strain, complete genome sequences of the three reemerging Korean PEDV strains were determined using Sanger sequencing.

For Sanger sequencing, 18 primer pairs were designed for the highly conserved sites of the PEDV genome using Primer3

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Table 1
Primers used for the amplification of full-length genomes of the reemerging Korean PEDV strains.

Primer	Sequence (5′-3′)	PCR product size (bp)	Position
1F	CTTAAAAAGATTTTCTATCTA	394	42–436
1R	CCTCAGAATAGTATGAGACG		
2F	GTCGCCTTCTACATACTAGACAAACAGC	1542	237–1778
2R	CCGACCTTTAAGCAGTCACAGG		
3F	CTTGGGAGCAGCTTAAGGC	1600	1562–3161
3R	CCTGTAACCTTGATACGATTACCAACAGC		
4F	CTATTATGATGGAACACTATACTATCC	1755	2946–4700
4R	GCATCTACCAAGCCATCC		
5F	GGTCTTAAGGTCTTTAATGTTGTTGG	1934	4441–6374
5R	CCCAACGCCTTTGCATTATAGC		
6F	CAGACGGCTGTTGTGATTAAAGACC	1849	6103–7951
6R	CAGCAACTATGAACAGACACAAAAACC		
7F	TTTATGTTGACCTTTAATGATTGTCGTATGC	1833	7813–9645
7R	TCTAACTGTGCGATAGGTGTACTTAGG		
8F	CCTTTCTGTTTTACGCCTCC	1937	9495–11431
8R	GCTTAACCAACTGAGGTGG		
9F	TATGTTGCAGAGTGTTGC	1876	11313–13188
9R	TAACGCATTTAAGCATAGC		
10F	TTACCGAGTATACTATGATGG	1873	12973–14845
10R	ACGAACTGGTCATCGACG		
11F	ATGCAACCACCGCATATGC	1699	14671–16369
11R	AGTGAATCGACCGCTGC		
12F	AAGCTTATTCTAGCTTAGTGC	1833	16198–18030
12R	TTATGGCATCACCAGAAGC		
13F	TACTGTTGTTTCAAACATGC	1886	17874–19759
13R	AATGTCTGGAGTTTATGATCC		
14F	ACGAGTTTGTGTCTAGTAATGATAGC	2324	19531–21854
14R	TGACAGTAGGAGGTAAAACAGCC		
15F	ATGGAGTTTGTAATGGAGC	2092	21579–23670
15R	TCAGCAAGCAATTGCTGG		
16F	GGTGTCATGGTACTACCT	2156	23477–25632
16R	TAAACTGCGCTATTACACAACC		
17F	TTCAACTAGACGAGTATGC	1825	25469–27293
17R	AAGCTGCTACGCTATTTTCG		
18F	GATGATCTGGTGGCTGC	980	27071–28050
18R	GGTCTTCAGTTACTAACAGTCC		

(Koressaar and Remm, 2007; Untergasser et al., 2012) or designed manually when Primer3 failed to identify optimal primer sites near the appropriate genomic regions (Table 1). Eighteen DNA fragments, which covered the entire genome of PEDV except the noncoding regions from both ends, were amplified using the SuperScript® One-Step RT-PCR System. Sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The products were analyzed using ABI 3730xl DNA Analyzer (Applied Biosystems). The sequencing results were assembled using Geneious v5.6.7 software. Complete genome sequences of the three reemerging Korean PEDV strains have been submitted into the GeneBank database under the accession numbers KJ588062, KJ588063, and KJ588064.

Complete genome alignment between the reemerging Korean strains and ten other available strains were performed using Multiple Alignment with Fast Fourier Transformation (MAFFT) v6 (Katoh and Toh, 2008). To study the relationship between the US and Korean PEDV outbreaks, US and Chinese strains (which were epidemic in 2012) were included in genome alignment. In addition, all available complete genome sequences of PEDV isolates from Korea were included in the alignment to compare the recent Korean strains with previous endemic Korean PEDV strains. CV777 strain was included in the alignment as PEDV reference strain. The maximum likelihood phylogenetic trees for the complete genome and the S gene sequence alignments were generated using PhyML version 2.4.4 (Guindon and Gascuel, 2003) with the generalized time reversible (GTR) substitution model (Rodriguez et al., 1990). The best nucleotide substitution model for analysis was confirmed using MEGA 5.2.2 (Tamura et al., 2011).

3. Results and discussions

The complete genome sequences of the reemerging Korean strains showed a typical PEDV gene order of 5′UTR-ORF1a/1b-S-ORF3-E-M-N-3′UTR and were identical or almost identical (one synonymous single-nucleotide polymorphism (SNP) in the ORF1a/1b gene) to each other. Multiple alignment with other PEDV complete genomes indicated that the reemerging Korean strains possess genome sequences, which are distinct from those of previous Korean field strains (Fig. 1). A previous study had discussed evidence of frequent recombination events between different genetic lineages or sublineages of PEDV (Huang et al., 2013). However, genomic sequences of the reemerging Korean strains did not show any regions recombined with those of the previous Korean strains during the recombination analysis performed using SimPlot 3.5.1 (Lole et al., 1999) (data not shown). In addition, phylogenetic analysis of the S gene between the reemerging and previous Korean strains of PEDV indicated that the reemerging Korean strains were included into a genetic lineage different from those of previous endemic Korean PEDV strains (Fig. 2). These results suggest that the reemerging strains have been recently introduced into Korea from another country.

Interestingly, comparative genome analysis of the reemerging Korean PEDV isolates and other strains revealed that the complete genome sequences of the recent Korean strains were almost identical (99.9%) to those of the US PEDV strains isolated in 2013 (Fig. 1). Compared with the complete genome of the reemerging Korean isolates, genomes of the US strains, USA/Iowa/18984/2013 and USA/Indiana/17846/2013 showed five (three non-synonymous

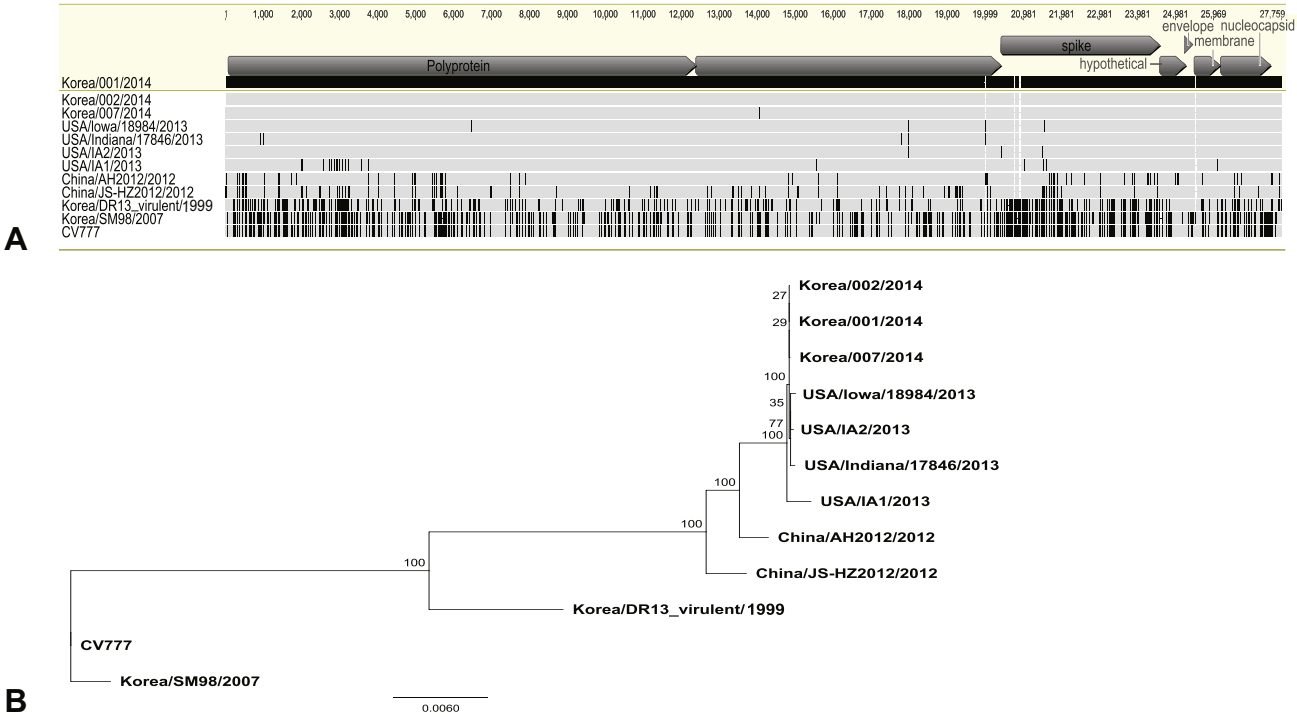

Fig. 1. Nucleotide sequence alignment and phylogenetic tree analysis for complete genomes of PEDV strains. (A) Alignment of the complete genome sequences of the reemerging Korean PEDV field strains and other strains was performed using MAFFT. One of the reemerging Korean strain sequences was set as the reference sequence. Vertical lines indicate the SNPs compared to the reference sequence and dashes indicate sequence gaps. Protein-coding regions are indicated with arrows. (B) A maximum likelihood phylogenetic tree was generated using the alignment. One-hundred bootstrap replicates were used to assess the significance of the tree topology. A bar indicates nucleotide substitutions per site.

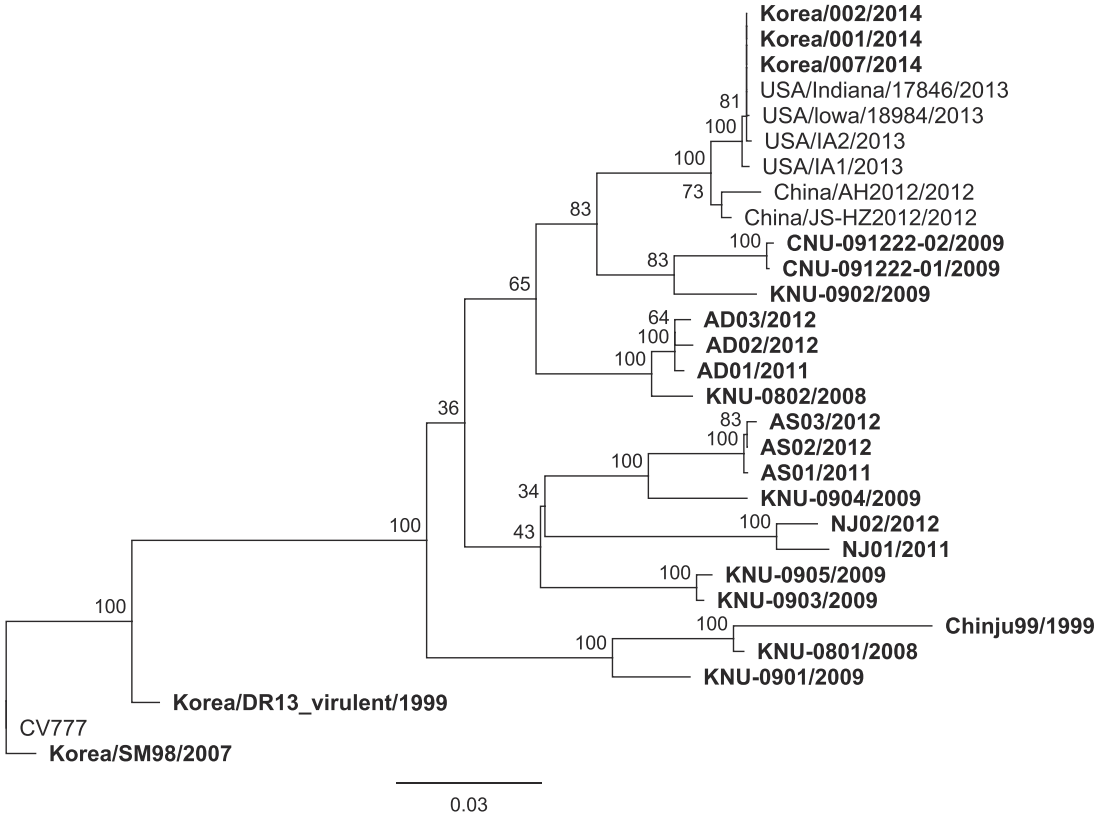

Fig. 2. Phylogenetic tree showing the relationship between the reemerging Korean strains from previous endemic Korean strains and strains from overseas, based on the analysis of the S gene. A maximum likelihood phylogenetic tree was generated from the alignment of complete S gene sequences. One-hundred bootstrap replicates were used to assess the significance of the tree topology. A bar indicates nucleotide substitutions per site. Korean PEDV strains are denoted using bold characters.

and one synonymous in the ORF1a/1b gene and one non-synonymous in the S gene) and seven (four non-synonymous and two synonymous in the ORF1a/1b gene and one non-synonymous in the ORF3 gene) SNPs, respectively. Both US strains have one insertion sequence causing early termination of the translation of polyprotein encoded in the ORF1a/1b gene. On the other hand, the complete genome of the US strain USA/IA2/2013 did not show any indels, but nine (three non-synonymous and three synonymous in the ORF1a/1b gene, one non-synonymous in the S gene, and one non-synonymous in the N gene) SNPs, when compared with the complete genome of the reemerging Korean isolates. According to the phylogenetic analysis, the reemerging Korean PEDV isolates were closely clustered with the US strains isolated in 2013 and Chinese strains isolated in 2012 (Fig. 1).

Comparative genome analysis and phylogenetic analysis revealed that the reemerging Korean PEDV strains are practically identical to the US strains. A previous study suggested that the three emergent US strains were most closely related to a strain isolated in 2012 from Anhui Province in China. In addition, the genomes of the reemerging Korean PEDV strains did not possess any genetic feature from the genomes of the previously sequenced Korean field and attenuated vaccine strains. These results suggest that the reemerging Korean PEDV strains are not variant strains of old Korean field or attenuated vaccine strains. There are two possible sources of origin of the reemerging Korean PEDV strains. First, the same source of origin of the US strains containing Chinese PEDV-like virus could have been introduced into Korea slightly later than the US outbreak events. This hypothesis can explain why the reemerging Korean PEDV strains are identical to the US strain. Another possibility is that US strain has been directly transmitted into Korea. During the US outbreak of PEDV in 2013, two genetic sublineages of the US strains were isolated. In a previous report, the authors stated that the US strain of PEDV diverged during evolution and that evolution generated two genetic sublineages, namely, IA1-CO/13 and MN-IA2 (Huang et al., 2013). During complete genome alignment as part of this study, one of the US strains, IA1, showed a recombined genomic region in the ORF1a/1b gene, which closely matched that of the Chinese strain JS-HZ2012. All reemerging Korean PEDV strains isolated in this study showed a close relationship with only one of the genetic sublineages of the US strains, namely, MN-IA2. We could not detect a PEDV strain with a close relationship with the IA1-CO/13 sublineage. This possibly suggests that only one sublineage of the US strain has been directly introduced into Korea from the US. To identify the exact source of origin of the reemerging Korean strain, further investigation and surveillance are required. Furthermore, to prevent the introduction of PEDV into Korea from overseas in future, the quarantine policy on feed ingredients should be reinforced.

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