Annotated sequence report

Molecular Characteristics of the Novel Recombinant of Porcine Epidemic Diarrhea Virus

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Abstract

Porcine epidemic diarrhea (PED) is a contagious viral disease in pigs, caused by coronavirus - porcine epidemic diarrhea virus (PEDV). PEDV results in significant mortality among piglets in non-vaccinated herds. Like many others RNA viruses, PEDV has high evolutionary rate and prone to genetic mutations. In this study, we characterized the complete genome sequence of the recently sequenced PEDV/Belgorod/dom/2008. The recombination event in S gene of PEDV/Belgorod/dom/2008 was detected. Pairwise identity analysis of the whole genome sequences revealed that PEDV/Belgorod/dom/2008 is an intermediate of PEDV and transmission gastroenteritis virus (TGEV) strains. The obtained results can be used for further analysis of the evolutionary variability, appearance and epidemiology of the porcine epidemic diarrhea virus.

Keywords

Coronaviridae, porcine epidemic diarrhea virus, recombination.

Text

Enteropathogenic porcine coronaviruses affect the animal's herds around the world leading to significant financial losses. Among them, porcine epidemic diarrhea (PED) is a highly contagious viral disease in pigs, caused by an RNA-containing virus belongs to the *Coronaviridae* family. PED is characterized by debilitating diarrhea, body dehydration and high mortality. The disease affects pigs of all age groups, but the most susceptible are newborn piglets (up to a two-week-old age) among which mortality ranges between 50-100% [1,2].

PED is common in the United States, Canada and more recently in China, Korea, Japan, Thailand and Vietnam and many other European Union countries with the exception of Ireland, Denmark and Sweden [3-7]. The PED was first introduced into large pig farms in the Russian Federation in 2006. At present, risk assessment notify

the incresing prevalence of PED within regions with high pig concentration in Russia. However, limited information is available about the genetic characteristics of PEDV strains currently circulating in Russia. All porcine epidemic diarrhea virus (PEDV) isolates form one serotype, but have different degree of virulence in the field [8].

The spike (S) protein of PEDV is a subject to the greatest immunological pressure and variability. Deletions (S-INDEL) or small insertions have been observed in the S gene nucleotide sequence of many PEDV isolates [9]. The PEDV strains that are currently circulating in the European Union are similar to the American S-INDEL strains [10-12]. The phylogenetic classification of the PEDV strains is based on the analysis of complete genomes sequences obtained worldwide [13] or individual genes such as S, M, N, or ORF3 [9, 11, 14].

In this study, we aim to analyze and further characterize the genome of recently sequenced PEDV isolate - PEDV/Belgorod/dom/2008 (GenBank accession number MF577027) [15].

Pathological samples (intestine, stomach) were taken from one-month-old sick piglets from Belgorod region of Russia in 2008 [16]. Total RNA was extracted from 10% organ suspension using TRIzol reagent (ThermoFisher Scientific) according to the manufacturer instruction. Next-generation sequencing was done with an Illumina MiSeq instrument with MiSeq reagent kit v3 in 2- × 300-bp PE mode (Illumina, San Diego, CA, USA) [15]. The PEDV/Belgorod/dom/2008 isolate was subsequently isolated from the small intestine tissue in Vero cell culture.

The prediction of homologous recombination events was carried out using the RDP4 (Recombination Detection Program) and SIMPLOT [17, 18]. Pairwise identity analysis was performed using SDT v1.2 software [19] and 18 whole genomic PEDV sequences, 3 TGEV sequences and swine enteric coronavirus strain from the GenBank database. Multiple alignment was performed using the MUSCLE software [20].

Phylogenetic trees were constructed based on PEDV M and S gene sequences using Maximum likelihood method in Mega 6.0. [21]. Bootstrap values were estimated for 1000 replicates.

The complete coding sequence of the PEDV/Belgorod/dom/2008 is 28,315 nucleotides (nt) in length (GenBank access number MF577027) [15]. Two recombination sites were detected on the recombinant PEDV/Belgorod/dom/2008 (Fig. 1). The recombination sites spans S gene of PEDV/Belgorod/dom/2008 in 20476 (ORF1B) – 24403 (S gene) nt. PEDV strain LZC (EF185992) and PEDV strain SLO/JH-11/2015 (KU297956) were identified as major and minor parental viruses, respectively. The recombinant event was identified by six modules (RDP, MaxChi, Chimaera, Geneconv, Bootscan, SiScan) with high confidence (Av. p-value 2,77 x 10^{-23}).

The similarity plot revealed the overall homology between the PEDV/Belgorod/dom/2008 strain and parental PEDV genomes, while there is a marked drop in the nucleotide similarity in the S gene region (Fig. 1).

Phylogenetic analysis of the complete genomes showed that PEDV/Belgorod/dom/2008 had a distant relationship to the known PEDV strains. PEDV/Belgorod/dom/2008 isolate does not belong to any groups formed by the American or Chinese strains and forms a separate cluster together with the SeCoV-ITA09 recombinant strain isolated in Italy (Fig.2).

Since only M gene PEDV sequences are available in the GeneBank for the Russian isolates, we rebuilt phylogenetic tree to refine the analysis. Based on the phylogenetic analysis of the M gene, PEDV/Belgorod/dom/2008 isolate belongs to the same clade as other Russian PEDV virulent strains, indicating a high sequence homogeneity in the M gene (Fig. 3 a). Interestingly, PEDV/Belgorod/dom/2008 carries significant number of nucleotide substitutions in comparison with the PEDV isolate Belgorod/05/07 (EU179730), isolated earlier from the same region.

The S gene phylogeny of PEDV and related coronaviruses demonstrates that the PEDV/Belgorod/dom/2008 isolate is genetically distinct and does not belong to any group (Fig.3 b). This robust incongruence between the M and S gene based trees may be explained by the recombination event within PEDV/Belgorod/dom/2008 isolate genome. Such variability in viral genome can lead to the dramatic changes in viral virulence, pathogenicity and antigenicity.

Pairwise identity analysis based on the spike amino acid sequences revealed that PEDV/Belgorod/dom/2008 is intermediate of PEDV and TGEV and also distantly related to other PEDV strains (Fig. 4).

PEDV/Belgorod/dom/2008 has a unique sequence of spike protein and a low similarity with other PEDV isolates. Changes in the S glycoprotein gene play an important role since it underlies tissue tropism and PEDV virulence [5]. Preliminary animal trial study with PEDV/Belgorod/dom/2008 demonstrated high virulence of this recombinant for non-vaccinated suckling piglets [22].

Recombination event cannot be ruled out and it can be observed in cases when the pigs have been vaccinated / infected with a mixture of TGEV and PEDV. Also, it would not be surprising that such recombination event can be responsible for loss of the vaccines efficacy.

According to Boniotti et al., 2016, a virus possessing the TGEV genome sequence in which the S protein sequence was identical to that of the PEDV (SeCoV-ITA09) appeared [23]. This chimeric virus probably appeared due to recombination between TGEV and PEDV. Similar chimeric viruses were also found by other research groups in Germany [24] and Eastern Europe [25].

The genome sequencing of one PEDV isolates (CH / HNQX-3/14) from China shown that this strain appeared due to naturally occurring recombination of attenuated strains (CV777 and DR13) with the circulating field strain (CH / ZMDZY / 11). The

recombination occurred in the S, ORF3, N structural protein-coding region and the replicase ORF1a region [26].

The results of phylogenetic and recombination analysis revealed the discrepancy between the S gene sequence of PEDV/Belgorod/dom/2008 and the sequences of other isolates available in the GenBank. Our results indicate that PEDV/Belgorod/dom/2008 is a new recombinant strain. Interestingly, that PEDV/Belgorod/dom/2008 and SeCoV-ITA09 (recombinant strain from Italy) form a unique phylogenetic group.

In addition, pairwise identity analysis demonstrates that S gene amino acids of PEDV/Belgorod/dom/2008 shares 60% homology with S gene of other PEDV strains and 50% homology with TGEV strains. These data argue the intermediate position of PEDV/Belgorod/dom/2008 between TGEV and PEDV.

The obtained results of the presence of PEDV/Belgorod/dom/2008 recombination processes can be useful for further analysis of virus evolutionary variability, epidemiology and development of a new diagnostic gene-based assay for porcine epidemic diarrhea virus.

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Conflict of interest. The authors declare no competing interests.

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Figure legends

Fig. 1. The scheme of recombination breakpoints of PEDV/Belgorod/dom/2008 isolate predicted by RDP4. The potential parent strains and recombinant isolate are shown in teal (major), purple (minor) and yellow (recombinant), respectively. Arrows indicate recombinant breakpoints. UTR, untranslated region; ORF, open reading frame; S, spike; E, envelope; M, membrane; N, nucleocapsid.

Fig. 2. The phylogenetic tree of the PEDV/Belgorod/dom/2008 isolate (highlighted in black) and other PEDV, TGEV and SeCoV strains of different geographical origin, compiled according to data on complete genome sequences based on amino acid alignment. The isolation year of LZC is unknown but should be before 2006 according to the GenBank submission date.

Fig. 3. Amino acid maximum likelihood phylogenies of the PEDV isolates and closely related coronaviruses (TGEV and swine enteric coronavirus strain). Phylogenetic trees based on M gene (a) and S gene (b) are presented. The bootstrap values equal or above 60 are shown close to the nodes. The trees describe robust incongruence for the PEDV/Belgorod/dom/2008 topology between M and S gene. The PEDV/Belgorod/dom/2008 is marked with black circle.

Fig. 4. Genome-wide pairwise identity matrix of the PEDV/Belgorod/dom/2008 and representative the spike amino acid PEDV and TGEV sequences. The PEDV/Belgorod/dom/2008 isolate is highlighted with black circle.

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