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Short communication

Insights into recombination-like events leading to outbreaks in USA through a retrospective study of porcine epidemic diarrhea virus isolates from China

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ABSTRACT

In April 2013, severe porcine epidemic diarrhea (PED) outbreaks first appeared in USA, causing significant financial losses. To detect the geographic origin and timing of USA porcine epidemic diarrhea virus (PEDV) isolates, we conducted a retrospective study to isolate 19 PEDV strains from positive samples obtained prior to April 2013. The results of phylogenetic and recombination analyses showed that GDS10 strain shared a common ancestor with CO13 strain, which was obtained from a piglet with severe diarrhea in USA.

1. Introduction

Porcine epidemic diarrhea (PED) is a highly contagious enteric disease of swine, featured with acute vomiting, dehydration, and watery diarrhea. All ages of swine are susceptible to the disease, however, the mortality is very high in young pigs, especially those younger than one-week old (Jung and Saif, 2015). Since its first discovery in the late 1970s (Pensaert and De, 1978), PED has continued to cause severe economic impact in swine industry worldwide. Before 2013, PED was prevalent in Asia and Europe. After the spring of 2013, PED outbreaks reached North America and spread explosively (Stevenson et al., 2013; Mole, 2013), causing to deaths of 7 million pigs across USA. Then Department of Agriculture of United States issued a Federal Order in June 2014 making swine enteric coronavirus diseases a reportable disease (http://www.aphis.usda.gov/aphis).

But where does the emergent strains of PEDV in USA came from? Plenty of investigations and researches were carried out to try to answer questions in terms of the epidemic following the initial outbreak of PED. Diep et al. reported that PEDV strains closely related to the initial US strains appeared in Northern Vietnam before the US PEDV outbreaks occurred based on the phylogenetic analysis of S gene/protein (Diep et al., 2017). The US MN PEDV strain shared 99.5% identity with China AH2012 strain, but which was not likely a direct source of infection according to genomic and phylogenetic analyses (Huang et al., 2013). The emergent US PEDV strain potentially descended from AH2012 and ZMDZY strains in G2b lineages through recombination (Tian et al., 2014). Other unidentified recombination events and accumulation of adapted mutations were likely involved in this process.

The key to detect the geographic origin of divergence of PEDV isolates lies in identifying the relatedness of viral whole genome sequences through retrospective testing. To fill this gap, we conducted a retrospective study to investigate molecular epidemiologic analyses using complete genome sequences of PEDV isolates obtained prior to the detection of PEDV in USA.

2. Materials and methods

123 positive samples of PEDV including feces and intestinal contents obtained prior to April 2013 were kept in our laboratories. These samples were originally collected from different farms in several provinces in China (Wen et al., 2018). 19 PEDV strains were isolated and propagated in Vero cells with 10 μg/mL trypsin from these positive samples as described previously (Hofmann and Wyler, 1988). PEDV was enriched for 100 folds and purified by sucrose density gradient centrifugation. The complete genome sequences of these virus strains were sequenced and assembled as described previously (Gong et al., 2017). Briefly, total RNAs of each sample were extracted using TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY, USA). cDNA was generated applying ProtoScript II reverse transcriptase and second strand synthesis enzyme mix (NEB, Ipswich, MA, USA). Size selection of adaptor-ligated DNA was then carried out using AxyPrep Mag PCR Clean-up (Axygen, Tewksbury, MA, USA), and fragments of ~360 bp

Abbreviation: Porcine epidemic diarrhea virus, PEDV

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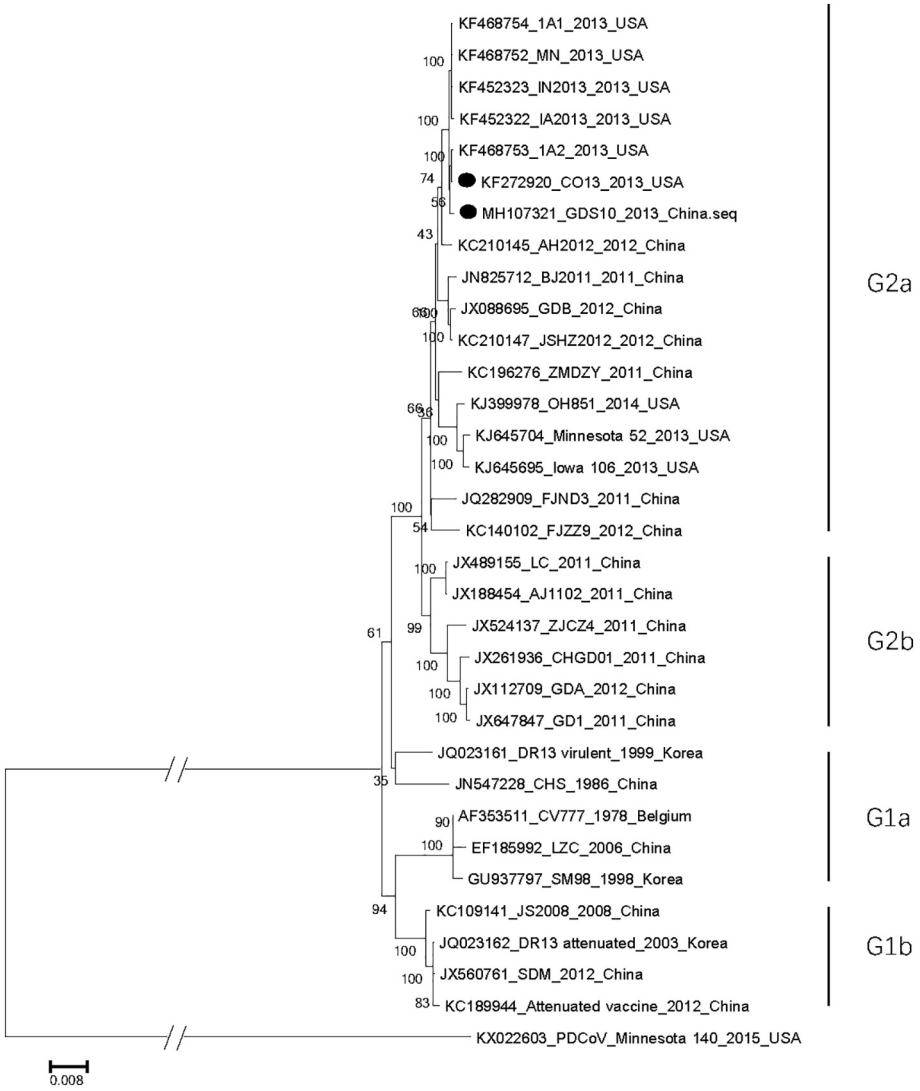

Fig. 1. Phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) strains based on complete genomic sequences. According to the full-length genomic nucleotide sequences, the tree was performed by neighbor-joining method using MEGA with 1000 bootstrap replicates. The porcine deltacoronavirus strain PDCoV/USA/Minnesota140/2015 sequence was used to set as an outgroup control. The names of GenBank accession numbers, the strains, years and places of isolation are show on the right in order, and the genogroups of virus are shown on the right of strains.

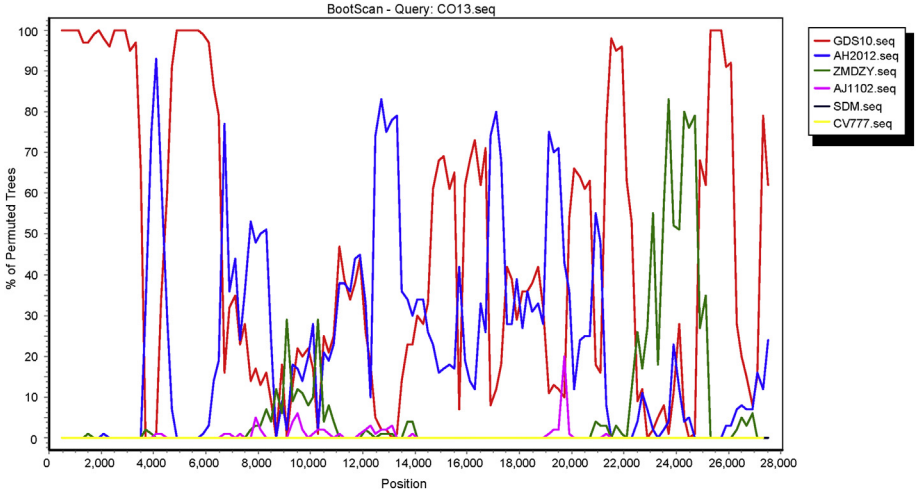

Fig. 2. Bootscan analysis for likely reconstruction events in USA. CO13 was set as the query group throughout the genome compared to new strain found in this study and represent strains in different genogroup. CV777, SDM and AJ1102 belong to G1a, G1b and G2b genogroup, respectively. G2a genogroup includes strains AH2012 and ZMDZY. The analysis was carried out by SimPlot (version 3.5.1; window size: 1000 bp; step, 200 bp).

(with the approximate insert size of 300 bp) were recovered. Sequencing was performed employing a 150 bp paired-end (PE) configuration in Illumina Hiseq 2500. Image analysis and base calling were performed by the HiSeq Control Software + OLB + GAPipeline-1.6 (Illumina, San Diego, CA, USA) on the HiSeq instrument. Alignment of complete genome sequences were performed using MAFFT. Phylogenetic analysis was conducted with neighbor-joining method using MEGA6 software. Bootscan analysis was used to detect the crossover points for the recombination events.

3. Results and discussion

After assembling and mapping sequenced reads, we obtained 19 complete genome sequences, of which GDS10 had a closest relationship with CO13 on phylogenetic tree and were deposited in GenBank (accession no. MH107321). GDS10 was isolated in Guangdong Province in 16 Jun 2013. The full genome length of GDS10 is 28,041 bp (excluding the poly-A tail). The S protein of GDS10 is 1 aa longer than that of CO13, which is the first reported sequence after PEDV being confirmed in the USA in April 2013 (Marthaler et al., 2013). Asp aa is inserted into S protein at the site of 232. GDS10 strain shares 99.9% sequence identity with CO13. GDS10 strain shares 100% aa identity with CO13 in E, M, N and ORF3 proteins, 99.9% in ORF1ab protein, and 99.5% in S protein. Phylogenetic tree based on the whole genome of GDS10, US PEDV strains, and all available strains obtained prior to the early of 2013 indicated that all PEDV strains could be divided into genogroup G1a, G1b, G2a, and G2b. All US strains clustered into 2 branches of G2a clade. 6 strains were the initial US strains and 3 strains contained insertions and deletion in the spike gene (S INDEL). These may be multiple PEDV strains being introduced into USA at the same or similar time. GDS10 clusters with the initial US strains more closely than AH2012 (Fig. 1), providing evidence of a common ancestor.

Divergence of coronaviruses is driven by genetic recombination (Hu et al., 2017). To accurately determine how the US strains are related to GDS10, we performed a recombination analysis with representative strains of different subtypes. Bootscan analysis suggested PEDV strains of G2a genotype were possibly conductive to diversifying new USA strains during the continuous progress of PEDV by potential reconstruction events while other subgroups were barely involved. As shown in Fig. 2, GDS10 and AH2012 recombined with CO13 in partial ORF1ab, 3′ half of S, ORF3, E, M and N, in partial ORF1ab, respectively.

In conclusion, we report the preliminary data on the phylogenetic and recombinant analyses of PEDV strain GDS10 with CO13 strain. GDS10 strain was more closely related to CO13 than AH2012, denoting GDS10 and CO13 shared a common ancestor. The determination of source of the emergent strains would help to explain the way by which the viruses could have been accessible to and infected USA pigs.

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