Novel Genetic Characterization of Porcine Epidemic Diarrhoea Virus Strains Circulating in Guangdong, China

Running Head: Novel Porcine Epidemic Diarrhoea Virus in China

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SUMMARY

In recent years, the emergence and high prevalence of porcine epidemic diarrhoea virus (PEDV) in piglets has been observed in various regio Province. In this study, novel genetic features of the PEDV S1 gene were detected in fifty-five PEDV samples from eleven different pig farms collected from 2018 to 2019 in eight cities Guangdong Province, China. More than 98.2% (54/55) samples tested positive with a PEDV antigen assay. The S1 gene of 11 samples was sequenced. Analysis results showed that PEDV isolates w 99.9%~100% identical to each other and clustered to a separate clade in the G subtype, sharing 90.4–98.8% and 87.7–97.7% identities at the nucleotide and amino acid levels, respectively, with 57 strains from GenBank. It is worth noting that the novel PEDV strains contained nine novel amino acid substitutions (L299I, V312A, Q319P, N/D360A, S558L, S566K, K593R, Y612H and S773F) in the S1 prote compared to the sequ widely used vaccine strains (CV777 and AJ110 strains) and other variant PEDV strains (G2 group strains). Furthermore, the amino acid substitutions were in the COE region of the important S protein neutralization epitope. In this study, we detected novel PEDV epidemic strains in G Province, which had the highest identity (98. nucleotide level, 96.6-97.2% amino acid level) with the BJ2011-1 strain and differed greatly from vaccine strains. Compared to vaccine strains, there are 91 (CV777) or 27 (AJ1102) an changes in the neutralization epitope of the S1 protein, and whether am substitutions affect the immune efficacy of the vaccine should be verified in further studies.

KEYWORDS: Porcine epidemic diarrhoea virus, Novel genetic diversity, S1 gene

Porcine epidemic diarrl (PEDV) is a large, enveloped, single-stranded positive-sense RNA virus with a 28 kb genome and is a member of tl Alphacoronavirus belonging to the family Coronaviridae of the order Nidov that can cause porcine epidemic diarrhoea (PED). PED is a highly contagious disease characterized by acute watery diarrhoea, vomiting, and dehydration, wi mortality ra, particularly in newborn pig(D. Sun, Wang, Wei, Chen, & Feng, 201. PED was first dis the 1970s in Enagn PEDV was subsequently identified as the causative agent, isolated, and designated CV7 Belgium in 197(Pensaert & de Bouck, 19′.{PEDV was the first outbreak in the 1970s in China. However, PEDV was first the 1980(Xuan et al., 1984.)Notably, temerging highly virulent PEDV caused massive outbreaks in southe Chin, leading to severe economic losses in the swine industry in 2010. The PEDV strains differ from the European strain (CV777) (Bi, Zeng, Xiao, Chen, & Fang, 2012; W. Li et al., 2012; R. Q. Sun et al., 201. Different types of PEDV are still common throughout China (Dong, Dai, Li, & Yang, 2020; Tan et al., 2020; Wen et al., 2018; Yu . However, variant PEDV strains (base on the S gene) remain the major cause of swine diarrhoea in Guang (Mai et al., 2018).

The spike (S) glycoprotein of PEDV, a major surface protein, contains two functional subunits: S1, responsible for cellular receptor binding, and S2 membrane fu(Liu et al.,. The S1 subunit consists of two domain namely, an N-terminal domain (NTD, residues 21–324 based on PEDV CV777) and a C-terminal domain (C(C. Li et al., 22 50 31–7 6, 3 18

2015. Moreover, the S1 subunit contains three neutralizing epitopes named COE, SS2, and SS6, and the regions of the aligned sequences that correspond to regions are amino acids 499–638, 748–755, and 76(C. Li et al., 2017; F. Li, 2015; D. Sun et al., 2008). Most of the PEDV strains have greater variability in the S1 gene, which has bee widely used for genetic relatedness and evolution as well as vaccine development (Chen et al., 2014; Jarvis et al., 2016).

In this study, fifty-five small intestine tissue samples from sick pigs suspected of PEDV viral infection were collected from eleven different swine farms in eight cities in Guangdong Province from 2018 to 2019 (Fig. S1). The sows were immunized with classical or other vaccine to prevent PED. However, 92.61% (2345/2532) of sick piglets exhibited multiple clinical symptoms consistent with previously reported PED (L. Wang, Byrum, & Zh, including severe enteritis, vomiting, a diarrhoea, dehydration, and mortality. A PEDV AG Test Kit (Bionote, Hwaseong, Korea) was used to detect PEDV in tissue samples, and t positive rate was 98.2% (54/55). We amplified the S1 gene to analyse mutations and evolutionary relationships with a previously described prc. (E. Wang et al., 2016). Then, the sequences of the S1 gene were cloned into the pCE2 vector using a 5 mi TA/Blunt-Zero Cloning Kit (Vazyme, Nanji). The recombinant plasmids were then sequenced in both directions by a commercial company using the Sanger method (Sangon, Shanghai, China). Representative sequences of S1 genes were randomly selected for naming CH/GD-4/2018 CH/GD-9/2018 (from Jiangmen), CH/GD-11/2018 (from Y 14/2018 (from Zhaoqing), which have been deposited in GenBank under accession numbers MT739790, MT739779, MT739781 and MT739784.

Comparison of S1 gene showed that CH/GD-4/2018, CH/GD-11/2018 and CH/GD-14/2018 shared 99.9% identity with each other and 90.4–98.8% and 87.7–97.7% identity at the nucleotide and amino acid levels, respectively. However, compared to the other 57 S1 genes, the background information is shown in phylogenetic trees (Fig. 1), which showed 90.4–98.8% and 87.7–97.7% identity at the nucleotide and amino acid levels, respectively. The detected Guangdong share 96.6-98% (nucleotide level) and 95.8-97.6% (amino acid level) identity with the S1 gene, indicating that the PEDV the trend of variation in Guangdong Province. Interestingly, the S1 genes of the four isolates shared 98.7-98.8% nucleotide identity with BJ-2011-1 from Beijing and 97.4-97% amino acid identity with AH2012 from Anhui Province. In addition, the four isolates showed low amino acid identity with two common vaccine strains, the classical attenuated strain CV777 (89.6-89.9%) and the variant strain AJ1102 (96.5%-96.8%).

Phylogenetic analysis, which was based on the complete S1 gene, was conducted with MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura using the neighbour-(Saitou & NNei, 1987 with 1,000 bootstrap replicates and a p-distance model. The results showed that CH/GD-9/2018, CH/GD-11/2018, and CH/GD-14/2018 clustered with the highly pathogenic mutant BJ-2011-1 and belonged to the PEDV G2a subgroup that is mainly endemic in Guangdong, which was consistent with previous (Fan et al., 2020; Gao et al., 2013; Mai et al., 201. Alignment of S1 protein amino acid sequences from the isolates with widely used vaccine strains and other variant PEDV strains revealed previously unreported amino acid substi L299I, V312A, Q319P, N/D360A, S558 L, S566K, K593R, Y612H and S773F (Fig.

2). In a recen study with amino acid variatic, the three-dimensional (3D) structure of the S1 protein cha (Su et al., 20. T) herefore, have modell, the 3D structure of the novel S1 protein, the open-source modelling server SWISS-MODEL (https://swissmodel.expasy.org/) from the Swiss Institute of Bioinformatics (Waterhouse et and the spike protein of PEDV (PDB ID: (Kirchdoerfer et al., as a templa Comparisoof these modelled tertiary structures were produced usi Pyth- ba molecular viewer PyMOL. The sequences involved in the modelling are G2a, G2b, AJ1102, CV777 CH/GD-4/2018, where G2a and G2b sequences are the main sequences of the G2a and G2b subgroups in the PEDV G2 group, respectively, and CH/GD-4/2018 is the representative sequence among the four isolates. The results showed that the isolates obtained in this study had three novel structural alterations in the NTD and CO regions compared to those of the reported PEDV strains (Fig. 3). The COE region is one of the important neutralizing ep (C. Li et al., 2, 0 a 7 whether these amino acid differences and alterations i protein structure have an impact on vaccine-induced immunity needs to be further determined.

In recent years, PEDV has been prevalent in various reg Provinc and has caused significant harm to piglets in the province. In this study, a novel PEDV strain was detected in newborn piglets with severe clinical symptoms from eight different cities. This strain was most similar to the BJ-2011-1 strain from Beijing in terms of nucleotide characteristics but had significant changes from the previously reported endemic strains and widely Guangdong, which altered the structure of the NTD and COE regions of the PEDV

S1 protein. In conclusion, the novel PEDV strain has enriched knowledge of the diversity of PEDV in Guangdong Province, with alterations in its neutralization epitopes and a possible progressive Therefore, additional extensive epidemiological investigations are ne further studies are needed to determine whether variations in those amino acid sites have an impact on the immunological efficacy of the vaccines.

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ETHICS STATEMENT

The study design was approved by the ethics committee for animal experiments at the animal ethics committee of the College of Life Science and Engineering, Foshan University, Guangdong, China.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding the research, authorship, and/or publication of this article.

AVAILABILITY OF DATA AND MATERIALS

The data set supporting the conclusions of this article is available in GenBank.

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Tables

Table 1. PEDV sample information for Guangdong Province in 2018-2019.

Figures captions

Figure 1. Phylogenetic analysis of PEDV isolates and reference strains based on the S1 gene. NJ tree of S1 gene nucleotide sequences from PEDV strains. Black triangles represent PEDV strains from Guangdong Province identified in this study. The name, GenBank accession number, country and year of isolation of each PE are shown in the phylogenetic trees.

Figure 2. Alignment of the deduced amino acid sequences of the S1 gene.

Figure 3. Comparison of the 3D structure of the S1 subunits. G2a and G2b represent the main sequences of the PEDV G2a and G2b subgroups, respectively. T protein modelling of the PEDV CV777 strain is shown as surface and cartoon representation in green. The S1-CV777 of strain is shown as pink surface. The S1-COE of the CV777 strain is shown as a yellow surface. The S1 protein modelling of the CH/GD-4/2018 strain is shown as surface and cartoon representation in red. The S1 protein modelling of the AJ1102 strain is shown as a cartoon in cyan. The S1 protein modelling of the G2a strain is shown as a cartoon in purple. The S1 protein modelling of the G2b strain is shown as a cartoon in orange.

Supporting information

Figure S1. Geographical distribution of the sampling farms in Guangdong Province. GZ: Guangzhou (1 farm, 3 samples), JM: Jiangmeng (1 farm, 6 samples), YF: Yunfu (1 farm, 3 samples), ZQ: Zhaoqing (1 farm, 3 samples), Huizhou (2 farms, 18 samples), FS: Foshan (3 farms, 13 samples), YJ: Yangjiang (1 farm, 5 samples), QY: Qingyuan (1 farm, 4 samples).