Dynamic Investigation of Porcine Epidemic Diarrhea and Analysis Sequence of Spike Gene in South China over the Past Five Years

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A B S T R A C T

The study executed a large-scale molecular epidemiological investigation of porcine epidemic diarrhea (PED) and five-yearlong monitoring of nine swine herds with the outbreak of diarrhea in south China. Porcine epidemic diarrhea virus (PEDV) exists all year round with varying degrees of mortality to suckling piglets, the highest mortality was in 2011 and followed by 2012, the lowest mortality was in 2013, with the mortality showed an increasing trend year by year during 2014 to 2015. It was noteworthy that the pregnant sows assumed the highest morbidity, followed by nursing sow while the nursed piglets and the growing pigs had lower morbidity. The current epidemic strains in south China were divided into two groups, Group 1 and Group 2 by generating the phylogenetic tree of thirty-five spike (S) genes during 2011-2015. Group 1 had 6.92-7.21% mutation rate while Group 2 owned 4.18% mutation rate when compared with CV777. The strains in Group 2 felled into the same branch with the previous Chinese isolates from 2004 while the strains in Group 1 had a close relationship with the United States strain. Interestingly, clinical morbidity caused by two types of PEDV strains were quite different, strains in G2-2 caused relatively minor mild clinical manifestations, presenting a smaller mortality and shorter onset period, while strains in G1-2 and G1-3 is caused by an outbreak of acute infectious diseases, showed a larger mortality and longer period especially PEDV strains in G1-3. It was also indicated that the effect way to prevent PEDV should focus on reduce the morbidity of pregnant sows.

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INTRODUCTION

Porcine epidemic diarrhea (PED) was caused by porcine epidemic diarrhea virus (PEDV) which was characterized by watery diarrhea, vomiting, dehydration, and a high mortality rate among swine (Vui, 2014; Alvarez et al., 2016). PEDV is an enveloped, single-stranded RNA virus, belongs to the family Coronaviridae. The genome contained seven open reading frames (ORFs) that encode four structural proteins: spike (S), envelop (E), membrane (M), and nucleocapsid (N) respectively (Luo et al., 2012; Wei et al., 2012). Spike (S) was believed as an important determinant for PEDV biological properties (Sun M et al., 2015) and was essential for ascertaining the genetic relationships among PEDV isolates, as well as the association between PEDV viral function and genetic mutations (Chen et al., 2013; Jung et al., 2015).

PEDV was reported in United States in April 2013 firstly (Stevenson et al., 2013) and it has since been confirmed in Canada, Mexico, Germany, France, Switzerland, Hungary, Italy, and Vietnam (Song et al., 2006; Puranaveja et al., 2009; Chen et al., 2010; Park et al., 2011; Vui, 2014; Oppriessig, 2015; Scott et al., 2015; Alvarez et al., 2016). In China, PED was occurred before 2008(Chen et al., 2008). However, variant strains of PEDV associated with rapidly spread and large-scale outbreaks of diarrhea have emerged in China since Oct 2010, affecting pigs characterized by high mortality risks
among suckling piglets within 7 days and resulting in the death of >1,000,000 piglets (Sun et al., 2012), resulting a serious and continuous concern for the swine industry and significant economic losses in China. Most of the affected swine farms lost all of their newborn piglets which was distinguished from the previous ones which had been reported (Puranaveja et al., 2009). However, it was found relatively uncommon among weaners and growers, few of them showed any clinical signs during the outbreaks, this was the similar popular features with those in Korea and Thailand (Puranaveja et al., 2009; Song et al., 2013). Many reports had described the epidemiologic feature of porcine epidemic diarrhea in China. Sun RQ reported that the PEDV positive rate was 82.0% (105/128) in fecal and intestinal samples (Sun et al., 2012). The findings suggested that porcine epidemic diarrhea was very serious in China during Oct 2010 to 2012 (Chen et al., 2012; Li et al., 2012; Li et al., 2012; Pan et al., 2012; Yang et al., 2013). However, the performance of PED became less severity with reduced morbidity and mortality in the clinical since May 2013. Most pig farms did not appear with acute outbreaks of PED no longer, clinical signs became less and less. The reason why there was a huge conversion with the clinical of PED is not clear.

Even though popular features of PEDV had been investigated in China, there is a lack of knowledge about the dynamics of disease spread. In the study, a large scale dynamic investigation and five yearlong monitoring of 9 swine herds with the outbreak of diarrhea located in geographically separate regions in south China were conducted. The morbidity and mortality on affected farms were mainly determined. In addition, the full-length S gene of thirty-five PEDV field strains were determined to obtain the relationships and genetic diversity of PEDVs in south China over the past five years.

MATERIALS AND METHODS

Sampling: Porcine intestinal and fecal samples were collected between January 2011 and Jul 2015 from 18 swine farms in five provinces (Guangdong province, Guangxi province, Sichuan province, Jiangsu province) in south China with the piglets younger than 7 days old that showed watery diarrhea and dehydration. Each farm had more than 3000 breeding sows, in which management practices and hygienic conditions are generally satisfactory, most of the farms adopt an all-in-all-out system. The mortality of nine PEDV infection farms from January 2011 to Jul 2015 in south China were collected, which included suckling piglets, pregnant sows, nursing sows, nurset piglets and the growing pigs (Table 1).

Diagnosis of porcine epidemic diarrhea virus: The morbidity and mortality of the nine selected farms was accurate recorded by Office Software 2010. The disease was diagnosed by clinical symptoms first, and then the intestinal and fecal samples were sent to laboratory to confirm the presence of virus using duplex RT-PCR assay.

Determination of S genes: RNA was extracted from the supernatant using TRIzol Reagent (Invitrogen Corp, Carlsbad, CA, USA) following the manufacturer’s instructions. The primers were shown in Table 2. The S gene of PEDV-positive strains were analyzed and submitted to GenBank.

<table>
<thead>
<tr>
<th>Herd name</th>
<th>Geographic origin</th>
<th>Herd size</th>
<th>Use commercial vaccines</th>
<th>Management mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.1</td>
<td>Guangdong province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.2</td>
<td>Guangdong province</td>
<td>4000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.3</td>
<td>Sichuan province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.4</td>
<td>Guangxi province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.5</td>
<td>Jiangsu province</td>
<td>4000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.6</td>
<td>Guangdong province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.7</td>
<td>Guangxi province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.8</td>
<td>Fujian province</td>
<td>5000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
<tr>
<td>No.9</td>
<td>Guangdong province</td>
<td>4000</td>
<td>Yes</td>
<td>all-in-all-out system</td>
</tr>
</tbody>
</table>

**Table 2:** The primers used in our research

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Size and amp monogent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFT</td>
<td>5’-CATTTTGCTTCCTAAAAGTAC-3’</td>
<td>S gene (4152-4161 bp)</td>
</tr>
<tr>
<td>SR1</td>
<td>5’-AGACAGCATGACTGAATCCTTT-3’</td>
<td></td>
</tr>
<tr>
<td>SR2</td>
<td>5’-GTTCGAGCATGATTTGACACAA-3’</td>
<td></td>
</tr>
</tbody>
</table>

Sequence analysis and phylogenetic analysis of S genes: Nucleotide sequences were analyzed using the CLUSTALX v1.83, Biodes v7.0.5.2 programs, MegAlign software (MEGA, version3.0) and DNAStar for alignment and sequence analysis. Phylogenetic tree was constructed using molecular evolutionary genetics analysis (MEGA, version5.0) with the neighbor-joining (NJ) method. Bootstrap values were estimated for 1,000 replicates. The reference strains used for phylogenetic analysis with PEDV strains were described in Table 3.

RESULTS

Statistics of the mortality and morbidity in infected pig farms: The recorded data in Office Software 2010 showed that there was 50-100% mortality rate in sucking piglets in 2011 and 0-30% mortality rate during 2012 to 2015. PEDV exists all year round with varying degrees of mortality to sucking piglets, the highest mortality was in 2011 and followed by 2012, the lowest mortality was in 2013, with the mortality showed an increasing trend year by year during 2014 to2015. The curve range of morbidity in 2011 was consistent with the range 2012, 2013 and 2014. (Fig. 1). The performance of the nine farms among sucking piglets in 2011 was listed separately due to the high mortality. It was shown that PEDV outbreak among the whole year with the highest mortality in February that aroused in No.7 farm and No.1farm respectively. The mortality of the PEDV maintained in a high level until the August 2011 (Fig. 2).

In contrary, the outbreak of the PED brought mild impact on the nursing sows, nurset piglets and the growing pigs with morbidity ranged from 20-80%. The clinical signs of PEDV infected gilts and sows including anorexia, depression, agalactia, transient diastase, and watery diarrhea. The morbidity of pregnant sows, nursing sows, nurset piglets and the growing pigs were shown in Fig. 3. The pregnant sows assumed the highest morbidity, followed by nurset sow while the nurset piglets and the growing pigs had lower morbidity. It was noteworthy that the diarrhea epidemic caused the most significant impact on the pregnant sows with nearly one thousand sick pigs at No.7 farm in April 2012. It is interesting that morbidity of the pregnant sows was very low in February while the mortality of the piglets was very high in February.
Phylogenetic analysis of the S gene: Thirty-five S genes which were determined during 2011 to 2015 and thirty reference strains were selected to construct the phylogenetic tree on the basis of nucleotide and deduced amino acid sequences (Fig. 5). The result showed that all PEDV strains could be divided into two groups, Group 1 and Group 2. Group 1 have four subgroups, G1-1, G1-2, G1-3 and G1-4. Subgroup G1-1 comprises seven United States strains, five Taiwan strains and two south China strains; G1-4 comprises one Korean strain and one Chinese strain. Twenty-five south China strains isolated during 2011 to 2015, eight Chinese strains (isolated in 2012-2013) and one United States strain formed subgroup G1-2 and G1-3. It is remarkably that the subgroup G1-2 comprises most of the strains isolated in 2012-2015 while the subgroup G1-3 comprises most of the strains isolated in 2011. Group 2 have two subgroups, G2-1 and G2-2. Subgroup G2-1 contains PEDV isolates that included three previous strains and vaccine strain; Subgroup G2-2 comprises nine Chinese strains, one of them isolated in 2004 (JS-2004-2) and eight else isolated 2011-2015 in our study. It was also found that among thirty-five south China strains, twenty-five of them have a close relationship with the United States strain (OH851-5), two of them have a close relationship with the United States strains and Taiwan strains while eight else were felled into the same branch with the previous Chinese isolates from 2004.

Sequence homology analysis of the S gene: Sequence homology results showed that the south China strains shared 93.0-100% nucleotide sequence identity with each other and 92.4-99.0% with the thirty reference strains reported in GenBank respectively. PEDVs in Group 1 have 97.5-100% nucleotide sequence identity with each other, and they have 93.7-95.9% sequence identity with the strains in Group 2. More precisely, eight south China strains and the reference strains. Details of the strains and the reference strains used in this study (Table 3).
strains in G2-2 have lower (94.8-95.7%) sequence identities with other twenty-seven Chinese field PEDV strains in subgroup G1-2 and G1-3. The subgroups G1-2 and G1-2 have 97.9% -98.7% homologies with each other. According to the south China strains property analysis, eight strains in G2-2 had lower identity to other south China strains in subgroup G1-2 and G1-3, whereas the strains had higher sequence identity with early reference strain isolated in 2004 (JS-2004-2). These results indicated that there were at least two types of PEDV strains existing in south China. Interestingly, clinical morbidity caused by two types of PEDV strains were quite different, strains in G2-2 caused relatively minor mild clinical manifestations, presenting a smaller mortality and shorter onset period, while strains in G1-2 and G1-3 is caused by an outbreak of acute infectious diseases, showed a larger mortality and longer period especially PEDV strains in G1-3.

**DISCUSSION**

Since its emergence in late 2010, PEDV has continued to cause huge damage related to the economic and management in the swine industry in China (Sun et al., 2012; Wang et al., 2013; Sun et al., 2015). Although several control methods including vaccination and strict biosecurity have been implemented, several herds continue to experience repeated outbreaks (Lee et al., 2015; Opiressnig, 2015). The disease has developed to an endemic stage that has led to the necessity for further investigation into the genetic diversity of PEDV which may facilitate the development of a more successful control program and vaccines.

From five yearlong monitoring, it showed that the number of piglets died were enormous and the economic losses were significant while the impact of the health of other pig groups were not as severe as the impact on piglets to some degrees and the economic losses were less profound on other pig groups during this epidemic of PEDV. The result was consistent with national reports in 2011-2013 (Pan et al., 2012; Sun et al., 2012; Yang et al., 2013).

It was noteworthy that the pregnant sows assumed the highest morbidity (for example, the diarrhea epidemic caused the most significant impact on the pregnant sows with nearly one thousand sick pigs at No.7 farm in April 2012), followed by nursing sow while the nursed piglets and the growing pigs had lower morbidity. It was indicated that the effect way to prevent PEDV should focus on reduce the morbidity of pregnant sows. Jung (Jung et al., 2015) also indicated that prevention and control measures to reduce the impact of PEDV during epidemics should focus on pregnant sows mainly to decrease the mortality in suckling pigs. The active immunization in growing pigs was crucial to prevention the endemic infection of PEDV.

By generating the phylogenetic tree, some characters of prevalent PEDV strains in this diarrhea epidemic were found. The current epidemic strains are divided into two groups, Group 1 and Group 2. The two types of the PEDVs had unique genetic features from the genetic analysis of the S genes. Group 2 had 4.18% mutation rate while Group 1 owned 6.92%-7.21% mutation rate when compared with CV777. Group 2 felled into the same branch with the previous Chinese strains while Group 1 had a close relationship with the United States strain. Between the two types of the PEDV strains identified in China 2012-2015, Group 1 has a closely relationship to US strains reported in 2013, whereas the precise propagation path related to the introduction of PEDV into America remains undetermined (Jung et al., 2015; Scott et al., 2015; Sun et al., 2015). Interestingly, clinically morbidity caused by two types of PEDV strains were quite different, strains in G2-2 (the farm number was No.6, No.4 and No.9) caused relatively minor mild clinical manifestations, presenting a smaller mortality and shorter onset period, while strains in G1-2 and G1-3 is caused by an outbreak of acute infectious diseases, showed a larger mortality and longer period especially PEDV strains in G1-3. Could we conclude, Group 1 and Group 2 (the farm number was No.1, No.3, No.7, et al.) on behalf of the highly pathogenic strains and the low pathogenic strains during the PEDV epidemic during 2011-2015 in south China respectively?

**Fig 1:** The mortality of piglets in nine farms during 2011 to 2015

**Fig 2:** The mortality of piglets in nine farms in 2011

**Fig 3:** The morbidity of pregnant sows, nursing sows, nursed piglets and the growing pigs in 2011.
Fig. 4: Phylogenetic analysis by neighbor-joining method based on nucleotide sequences of full-length S genes of 35 field isolates of porcine epidemic diarrhea virus (PEDV) in south China and other PEDV reference strains. The 35 field isolates in this study are marked by solid triangle symbols.

It is notably that most of the strains isolated in 2012-2015 distributed in the subgroup G1-2 while most of the strains isolated in 2011 distributed in subgroup G1-3. This was consistent with the reports in United States (Jung et al., 2014; Sun et al., 2015). The two subgroups G1-2 and G1-3 have 97.9%-98.7% homologies with each other. It is showed that the PEDV strains had some minor variations from 2011 to 2015. Chen JF confirmed that PEDV had a high detection rate which indicated the S genes were heterogeneous in China (Chen et al., 2013).
Pan also reported that PEDV strains prevalent in China was the new variant (Pan et al., 2012), which brought a certain degree of difficulty to choose the vaccine strain to prevent PEDV epidemic.

Although vaccination has been encouraged to use to induce specific immunity to PEDV in all pigs and to prevent the virus spread and epidemic, it seemed that vaccination against PEDV did not induce mucosal immune responses and effective protection, at least did not provide lifelong protection from PEDV as pigs in the same farm suffered re-infection within one year. The epidemic lasted for a long time and relapsed several times (Lee et al., 2015; Sun et al., 2015). Massive feedback to the pregnant sows using piglet faeces or minced piglet gut was performed in most of swine farms during our study with the aims to pass the sow’s protective immunity to the piglets. However, the effect was varied from farm to farm. The continuing monitoring of PEDV isolates in diarrhea farms has helped us to gain new insight of the biology of these viruses and will contribute to the development of effective vaccine to control the spread of PED.

Conclusions: In the study, the molecular epidemiological investigation and five yearlong monitoring of nine swine herds with the outbreak of diarrhea located in geographically separate regions of south China was executed. All S genes in the study could be divided into two types, the ones had 96 amino acids different when compared with CV777 and the mutation rate was 6.92% while the others’ mutation rate was 4.18%. The amino acids variations of the former were identical to the United States strains and Taiwan strains. The two types on behalf of the highly pathogenic strains with acute virulent, caused significant economic loss and the low pathogenic strains caused a relative moderate diarrhea in clinical with less economic losses in this PEDV epidemic during 2011-2015 in south China. It was also indicated that the effect way to prevent PEDV should focus on reduce the morbidity of pregnant sows.

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Author’s contribution: ZL, ZL and LC conceived and designed the study. YC, XZ, BS, QX, XL and HG executed the experiment. JYM and YB analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES


