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High genetic diversity of porcine sapoviruses infection in Henan Province, China

Xiaochun Wang1§, Hua Tian2§, Yu Ling1, Xingli Fu1, Zeyu Li1, Xiaoying Zhao1, Xin Geng1, Shiji Deng1, Wen Zhang1, Jianqiang Wang3 and Quan Shen1*

1School of Medicine, Jiangsu University, Zhenjiang, Jiangsu 212013, China.
2Jiangsu Provincial Center for Disease Prevention and Control, Nanjing, Jiangsu 210009, China.
3No. 2 People's Hospital of Jintan City, Changzhou, Jiangsu 213200, China.

§These authors contributed equally.

*Corresponding author. E-mail: shenquan@ujs.edu.cn, Tel: 86-511-85038449.

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Sapoviruses (SaVs) belong to the family of Caliciviridae and are related to gastroenteritis of humans and animals worldwide. New strains or genogroups are emerging globally, however the related data of China are limited. To determine the prevalence and genogroup distribution of SaVs in Henan area, central China, 169 pig fecal samples were screened by RT-PCR method. Results showed that the overall positive rate of SaVs was 8.9%. Phylogenetic analysis indicated that these SaVs strains clustered into three reported genogroups; GIII, GVII, GVIII, and two new genogroups; GIX and GX. To the best of our knowledge, this is the first report of high genetic diversity of porcine SaVs infection in China including two new genogroups.

Key words: Porcine sapoviruses, genetic diversity, prevalence.

INTRODUCTION

The prototype of Sapoviruses (SaVs) was first identified in 1977 by electron microscopy of fecal specimens from children with gastroenteritis in the city of Sapporo, Japan (Flynn et al., 1988). SaVs now have been detected in different animal species including cattle, dog, bat and mink (Guo et al., 2001; Li et al., 2011; Mijovski et al., 2010). Currently, the porcine sapoviruses (PoSaVs) infection has been reported in both developed and developing countries including China (Chao et al., 2012; das Merces Hernandez et al., 2014; Shen et al., 2009; Wang et al., 2006). Moreover, some PoSaVs isolates, genetically closely related to human strains, were identified. Also, some potential recombinant SaVs have reportedly raised the possibility of zoonotic transmission (Dos Anjos et al., 2011; Guo et al., 1999; Li et al., 2017).

The genome of SaVs is 7.1 to 7.7 kb in length, typically including two open reading frames (ORFs; Chiba et al., 2000). ORF1 encodes a polyprotein that is cleaved by a viral protease to form nonstructural proteins and the major capsid protein (VP1). ORF2 encodes a small structural protein (VP2). SaVs have been classified into at least five genogroups (GI-GV). GI, GII, GIV and GV cause gastroenteritis in humans, while GIII viruses infect pigs (Oka et al., 2016). Recently, some new genogroups including GIX and GX were identified in some countries (Reuter et al., 2010; Scheuer et al., 2013). To date, only GIII viruses were detected in swine of China.

Since SaVs are highly diverse, with new strains and genogroups continually emerging, there is a potential interspecies transmission; hence, it is important to monitor the prevalence and diversity of these viruses in swine (Scheuer et al., 2013). In previous research, the study reported the first porcine SaVs infection that led to an outbreak of gastroenteritis, and also evaluated the molecular prevalence of porcine caliciviruses in Eastern China (Shen et al., 2009; Zhang et al., 2008). The
objectives of current study were to test the recent prevalence and genetic diversity of SaVs in pigs in Central China swine farms and to investigate whether there are new genogroups or genotypes emerging in swine populations in this area.

MATERIALS AND METHODS

Sample collection

A total of 169 fecal samples from healthy pigs of various ages were collected from 4 swine farms from January to February 2015 in four different districts of Henan Province in Central China. The pigs were 3-5 months old, and fecal samples were collected manually from each animal. Fresh fecal samples were placed into sterile containers and stored frozen at -70°C until tested. Before test, fecal samples were converted to 10% (w/v) suspensions in phosphate-buffered saline (PBS). The total ribonucleic acid (RNAs) were extracted and purified from 200 μl of clarified stool suspensions using the TaKaRa MiniBEST Viral RNA/DNA Extraction Kit according to the manufacturer’s instructions (TaKaRa, China). RNAs were eluted in 30 μl of RNase-free water, and reverse transcription (RT) was performed immediately.

RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) was performed separately using primer pair p290 and p289 as previously reported to detect members of the two genera of Calicivirus: SaVs and norovirus (NoVs) (Jiang et al., 1999). RT was performed in 10 μl reaction mixture containing 2 μl of RT buffer, 0.5 μl (200 units) of AMV reverse transcriptase (TaKaRa, China), 1 μl (25 mM) of reverse primer and 6.5 μl extracted RNA at 42°C for 1 h. PCR assays were carried out using 5 μl of the synthesized cDNA with ExTaq DNA polymerase (TaKaRa, China) for 35 cycles. The amplicons were analyzed by 1.5% agarose gel electrophoresis in TAE buffer, followed by staining with ethidium bromide (0.5 μg/ml) and visualized under UV light.

DNA cloning and sequence analysis

RT-PCR products were purified using QIAquick gel extraction kit (Qiagen, Germany) following manufacturer's instructions. The purified PCR products were ligated into pMD18-T vector (TaKaRa, China) using T4 DNA ligase (TaKaRa, China). For each sample, four clones were sequenced by Life Technologies (Shanghai, China), using a BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Life Technologies, China) with the M13 Forward Sequencing Primer. Basic Local Alignment Search (BLAST; http://www.ncbi.nlm.nih.gov) were carried out with default values to find homologous hits (Altschul et al., 1997). Multiple sequence alignment was done by CLUSTAL W (version 1.8). Phylogenetic analysis (neighbor-joining) with bootstrap (1,000 replicates) was conducted using MEGA 6.0 (Tamura et al., 2013). For SaVs classification, 43 reference SaV sequences including 10 genogroups (GI-GX) were obtained from the GenBank database and the remaining 10 were from the current study.

RESULTS

A total of 15 samples from 169 pigs yielded a PCR fragment with the expected size (309-332bp) following RT-PCR (Table 1). All of the PCR fragments were confirmed as SaVs by sequencing. Neither NoVs infection nor co-infection of different SaVs strains is found in this study. Farms located in Anyang, Pingdingshan and Zhoukou were positive for SaVs and the positive rates were 12.8, 15.6 and 7.1%, respectively. SaVs were not detected in the samples collected from Zhengzhou. For simplicity, SaVs strains with identical nucleotide sequences were treated as a unique strain in the analysis, leaving a total of 10 strains with distinct sequences. Phylogenetic analysis based on the partial sequences of the RdRp gene indicated that these SaVs were divided into five distinct genogroups; GIll, GIVII, GVIII, and two new genogroups GIX and GX (Figure 1). Among the 10 SaVs strains in the present study, four were grouped into GIll, three belonged to GIVII, and the rest three strains were GVIII, GIX, and GX, respectively. Except Zhoukou, in all of the rest farms there were more than one genogroups SaVs which were detected, and

Table 1. Genogroup distribution and prevalence of SaVs detected by RT-PCR assay in fecal samples from four farms in Henan province, Central China.

<table>
<thead>
<tr>
<th>Farm location</th>
<th>No. of Samples tested</th>
<th>No. of positive</th>
<th>Prevalence (%)</th>
<th>Genogroups (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anyang</td>
<td>39</td>
<td>5</td>
<td>12.8</td>
<td>GIII (4), GIVII (2)</td>
</tr>
<tr>
<td>Pingdingshan</td>
<td>45</td>
<td>7</td>
<td>15.6</td>
<td>GIII (3), GIVII (1),</td>
</tr>
<tr>
<td>Zhoukou</td>
<td>42</td>
<td>3</td>
<td>7.1</td>
<td>GVIII (1), GX (2)</td>
</tr>
<tr>
<td>Zhengzhou</td>
<td>43</td>
<td>0</td>
<td>0.0</td>
<td>GIII (2), GIX (2)</td>
</tr>
</tbody>
</table>
notably, in Pingdingshan there were as much as four genogroups that were found. Meanwhile, Pingdingshan and Zhoukou had the highest or lowest infection rate, respectively. Based on phylogenetic analysis and alignment, strains SaV-HN2 (GenBank accession numbers: KT895957), SaV-HN5 (GenBank accession no.: KT895952), SaV-HN63 (GenBank accession no.: KT895959) and SaV-HN104 (GenBank accession no.: KT895961) were grouped into GIII, shared 79-99% nucleotide homologies among themselves. Strains SaV-
Table 2. Summary of the nucleotide sequence identities in the partial RdRp region between SaVs strains detected in this study and their closest strains.

<table>
<thead>
<tr>
<th>Identified strain</th>
<th>Strain Genebank accession no. Genogroup</th>
<th>Closest sapovirus Strain Genebank accession no. Genogroup</th>
<th>Percentage Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaV-HN2</td>
<td>swine/C28/CHN/2009 HQ292717 GIII</td>
<td>SaV-HN2 swine/C28/CHN/2009 HQ292717 GIII</td>
<td>95</td>
</tr>
<tr>
<td>SaV-HN63</td>
<td>sw/PC47b/NL JN644276 GIII</td>
<td>SaV-HN63 sw/PC47b/NL JN644276 GIII</td>
<td>90</td>
</tr>
<tr>
<td>SaV-HN104</td>
<td>sw/PC47b/NL JN644276 GIII</td>
<td>SaV-HN104 sw/PC47b/NL JN644276 GIII</td>
<td>91</td>
</tr>
<tr>
<td>SaV-HN19</td>
<td>swine/SUI-22/2008/PA/BRA KF241957 GVII</td>
<td>SaV-HN19 swine/SUI-22/2008/PA/BRA KF241957 GVII</td>
<td>92</td>
</tr>
<tr>
<td>SaV-HN45</td>
<td>swine/TYMPo155/08/JP AB521786 GVII</td>
<td>SaV-HN45 swine/TYMPo155/08/JP AB521786 GVII</td>
<td>85</td>
</tr>
<tr>
<td>SaV-HN59</td>
<td>SWECIII/VA112 AY615814 GVIII</td>
<td>SaV-HN59 SWECIII/VA112 AY615814 GVIII</td>
<td>87</td>
</tr>
<tr>
<td>SaV-HN60</td>
<td>Po/B2015/Brazil DQ359041 GX</td>
<td>SaV-HN60 Po/B2015/Brazil DQ359041 GX</td>
<td>91</td>
</tr>
</tbody>
</table>

HN18 (GenBank accession no.: KT895953), SaV-HN19 (GenBank accession no.: KT895954) and SaV-HN45 (GenBank accession no.: KT895955) clustered with GIVII, with 76-78% nucleotide homologies with each other. Strain SaV-HN59 (GenBank accession no.: KT895956) belonged to GVIII and clustered with a Netherlands strain (AY615814), sharing 87% nucleotide homology with it. Strain SaV-HN90 (GenBank accession no.: KT895960) clustered with a Belgian GIX strain PC29 (EU652845), sharing 90% nucleotide homology with it. Strain SaV-HN60 (GenBank accession no.: KT895958) clustered with a Brazilian GX strain B2015 (DQ359041), sharing 87% nucleotide homology with it. Nucleotide identities in the partial RdRp region between SaVs strains detected in this study and their closest strains were 85-95% (Table 2).

DISCUSSION

In the current study, SaVs were detected in farms located in the three cities except Zhengzhou, the capital city of Henan province with higher socio-economic level. The farms in this area have cleaner environment and stricter monitoring system, which suggests that stricter monitoring system should be helpful to prevent the infection of these viruses. In our previous research, we evaluated the prevalence of porcine CVs in finisher swine in Eastern China and found that the overall infection rate was 0.9% (Shen et al., 2009). Meanwhile, we did not detect SaVs infection in finisher pigs in Guizhou, a province in Southwest China (Shen et al., 2011). Although, some researches showed higher infection rates (6.9-14.37%), the target groups they screened were piglets with or without symptoms of diarrhea, respectively (Liu et al., 2012, 2014), because researches indicated that the infection rates in piglets were higher than that in finisher pigs (Martinez et al., 2006; Wang et al., 2006). In the current study, the overall infection rate was 8.9%, much higher than the previous studies of China. The high prevalence in finisher pigs of China increasingly raises public health concerns of these viruses. Some more systemic investigations on SaVs in the different areas of China should be carried out in the future.

GIII strains not only showed higher infection rate than the other genogroups, but also was detected in all of the three farms with positive samples. Recently, Reuter et al. (2010) found that GIII was still the predominant genogroup with the highest infection rate though five more genogroups; VI, VII, VIII, and potential new genogroups IX and X were identified. Moreover, in the previous studies, all porcine SaVs strains identified in China belonged to GIII except a GVII strain, suggesting that GIII is still the predominant genogroup worldwide. Nevertheless, the emergency of new genogroups of SaVs in Chinese porcine farms also raises the concerns on the prevalence of new SaVs.

Five genogroups of viruses that were detected in this study reinforced the high genetic diversity of SaVs in China. Moreover, the two new genogroups that was firstly found in China in the current study shared the highest nucleotide homology with two foreign strains. Combined, these results suggested that the variety of strains is circulating worldwide with the movement of animals and animal products in the different countries.

Porcine NoVs have been reported in different countries and regions and the prevalence varied from 51.8 to 0.5% (Chao et al., 2012; Di Bartolo et al., 2014; Silva et al., 2015). Some porcine NoVs are genetically and antigenically related to human NoVs and therefore pose a potential risk of interspecies transmission between pigs and humans (Wang et al., 2005). No NoVs infection that was detected in the current study suggested that porcine NoVs were not predominant enterovirus in China.

Conclusion

In summary, high genetic diversity of porcine SaVs clustered into five genogroups including two potential
new that was firstly detected in China were circling in central China. SaVs should continue to be monitored to elucidate their epidemiology and characterize newly emerging strains, so as to be helpful to control and prevent the spread of these viruses.

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Compliance

The authors declare that they have no conflict of interest. The guidelines pertaining to care and welfare of laboratory animals were fully abided by the Animal Care and Use Committee of Jiangsu University.

Authors’ contributions

QS and JQW conceived the study and designed the experiments. XCW, HT, YL, XLF, ZYL conducted the experiments. XYZ and XG analyzed data. QS drafted the final manuscript with the help of WZ and ZLR. All authors read and approved the final manuscript.

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