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Prevalence of Porcine Sapovirus in Suckling Piglets in Hunan Province, China

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Abstract

Sapoviruses (SaVs) are emerging enteric pathogens that cause diarrhea in humans and animals. In the current study, genetic identification of sapovirus (SaV) in suckling piglets was investigated in Hunan province, China between May 2013 and May 2014. Diarrheic fecal (n=300) samples collected from suckling piglets in Hunan province, China were evaluated using RT-PCR and then sequenced. Overall, 45 of the 300 (15%) samples were found to contain porcine SaV. In addition, phylogenetic analysis showed that all the porcine SaV isolates belonged to the porcine SaV genogroup III (GIII). The results of the present investigation have implications for the control of porcine SaV infection in pigs in Hunan province, China.

Keywords: China, pig, phylogenetic analysis, porcine sapovirus, prevalence

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Introduction

Sapoviruses (SaVs) belong to the family *Caliciviridae*. They are small (27-40 nm in diameter), non-enveloped viruses with icosahedral symmetry. The genome contains two main open reading frames (ORFs) and is about 7.4-8.3 kb in size (L'Homme et al., 2009). For sapovirus (SaV), ORF1 encodes the non-structural proteins as well as the capsid protein VP1; ORF2 encodes the minor and poorly characterised structural protein VP2 (Bank-Wolf et al., 2010). Based on VP1 sequences, SaVs are currently divided into five genogroups (GI to GV) (Farkas et al., 2004). Porcine SaVs belong to GIII (L'Homme et al., 2009), whereas human SaVs belong to GI, GII, GIV and GV. Like other RNA viruses, SaVs show broad genomic sequence diversity among circulating strains. RNA dependent RNA polymerase (RdRp) of SaV is a good proxy for characterization of *caliciviruses* (generally well conserved among the SaV genotypes) (Cunha et al., 2010), although ORF1/ORF2 junction region recombination events are very common. In addition, primers targeting the capsid region are also adequate for classification of SaV into their respective genotypes (Farkas et al., 2004).

Porcine SaVs were first identified in fecal specimens of piglets in the United States (Saif et al., 1980). To date, porcine SaVs have been reported in many countries or regions, including China (Phan et al., 2007). More importantly, SaVs are also considered as a major cause of acute gastroenteritis in young children in many countries or regions (Bank-Wolf et al., 2010). Although SaVs are responsible for significant public health problems and economic losses worldwide, to date, no vaccine is currently available and treatment is primarily limited to symptomatic measures (Bank-Wolf et al., 2010). In the People's Republic of China, the pig industry constitutes an important sector of agriculture and economic growth, and Hunan province represents currently one of the largest producer of intensively raised pigs in China. However, little information is available about SaV infection in pigs in Hunan province, China (Liu et al., 2012a,b). Importantly, previous studies have indicated that SaV infection is prevalent in weaning piglets and adult pigs in Hunan province (Liu et al., 2012a,b).

The objective of the present study was to determine the prevalence of SaV in suckling pigs in Hunan province, China. Genotyping of SaV using phylogenetic analysis based on sequences of Rdrp ORF was also conducted.

Materials and Methods

Collection and preparation of samples: A total of 300 diarrheic fecal samples were collected from 300 suckling piglets that were raised in 10 intensive farms (population more than 300) from ten representative administrative regions in Hunan province between May 2013 and May 2014 (Table 1). All the sampled animals were less than 4 weeks of age and were randomly selected from each herd. Before sampling, the pigs were subjected to clinical examination to determine their health status. Information about each pig such as age, medical history, growth hormones, and weight was collected. All the samples were labeled individually and cooled during transport to the laboratory, where the fecal samples were kept at 4°C for a maximum of 10 days before processing.

Table 1 Molecular prevalence of porcine sapovirus in suckling piglets in Hunan province, China

Locations	No. examined	No. positive	Prevalence (%)
Changsha	30	5	16.7
Hengyang	30	6	20
Yueyang	30	1	3.3
Yongzhou	30	5	16.7
Chenzhou	30	4	13.3
Loudi	30	3	10
Huaihua	30	8	26.7
Changde	30	3	10
Yiyang	30	7	23.3
Xiangtan	30	3	10
Total	300	45	15

RT-PCR for SaV detection: Viral RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from 200 µl of 10% fecal suspensions, according to the manufacturer's instructions. The total RNA pellet was suspended in 50 µl of RNase-free water and reverse transcription was performed immediately. The polymerase gene was amplified by PCR using previously reported primers (Li et al., 2012). PCR reactions (50 µl) were performed in 5 µl 10 x RT buffer, 1 µl (200 units) of AMV reverse transcriptase (TaKaRa, Japan), 1 µl (25 mM) of reverse primer, 4 µl (25 mM) of dNTPs, and 1 µl extracted RNA and by adding sterile H_2O to 50 µl in a thermocycler (Biometra) under the following conditions: after an initial denaturation at 94°C for 3 min, then a denaturation at 94°C for 30 s, an annealing at 57°C for 30 s, an extension at 72°C for 30 s for 30 cycles, followed by a final extension at 72°C for 7 min. The PCR products were purified from 1%

agarose gel using the QIAquick Gel Extraction kit (Qiagen, Gemany). Purified PCR products were ligated into pMD18-T Vector. For each product, three to five positive colonies were selected and sent to Sangon Company (Shanghai, China) for sequencing from both directions.

Sequencing and phylogenetic analysis: The partial polymerase gene sequences were aligned using the computer program Mafft-7.122 (Katoh and Standley, 2013). The polymerase gene sequences of representative samples were used for phylogenetic analyses. The polymerase gene sequences were aligned with reference sequences in the GenBank using Mafft-7.122, with manual adjustment. Phylogenetic relationship among the 28 SaV isolates (GI-GVIII) plus the 7 representative polymerase gene sequences obtained in the present study was reconstructed using one norovirus isolate (GenBank accession number AB126320: position 4692-5210) as the outgroup based on polymerase gene sequences. Bayesian inference (BI) was used for the phylogenetic analyses. BI was performed using MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003), and the GTR model with its parameter for the nucleotide dataset was determined for the BI using jModelTest (Posada, 2008) based on the Akaike information criterion (AIC). Two independent runs were performed for 1,000,000 metropolis-coupled MCMC generations, sampling a tree every 100 generation in MrBayes 3.1.1; the first 2,500 trees represented burn-in and the remaining trees were used to calculate Bayesian posterior probabilities (Bpp). Phylograms were drawn using the Tree View program version 1.65 (Page, 1996).

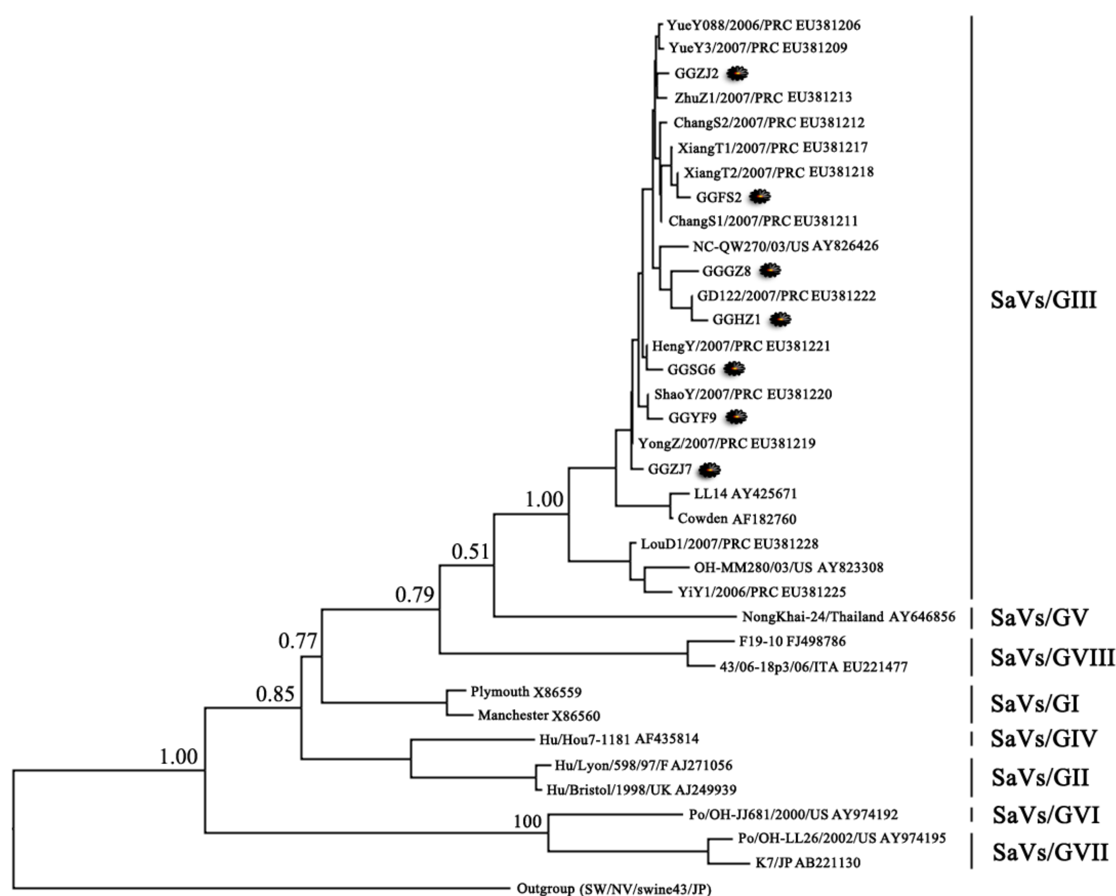

Figure 1 (Inferred phylogenetic relationship among sapoviruses based on analyses of polymerase gene sequences utilizing Bayesian inference using norovirus isolate as outgroup.

Results and Discussion

The partial polymerase gene amplicons of approximately 520 bp was subjected to agarose gel electrophoresis. No length variation was detected on the agarose gels among any of the amplicons examined (not shown). SaV sequences were found in 45 of the 300 (15%) fecal samples. The prevalence ranged from 3.3 to 26.7% (Table 1). The identity among all polymerase gene sequences was 86.3-91.6%. Sequence comparison revealed that the similarities in polymerase gene sequences among all isolates with that of the SaV available in GenBank (LL14, Cowden and SSSY1) were 78.7-96.1%. Sequence similarity among porcine SaV in China were 82.1-96.1%. To ascertain the identity of SaV isolates, the phylogenetic relationship of SaV was reconstructed (Fig 1). In this tree, within the SaV (GI-GVIII) clade, SaV/GIII and SaV/GV were more closely related than to other SaV genogroups. Within SaV/GIII, all the SaV isolates in Hunan province grouped with SaV/GIII, and with strong support (Bpp=1.0).

SaVs are known to cause gastroenteritis in humans and animals around the world. Although the prevalence or seroprevalence of SaV is present in pigs

in some provinces of China (Shen et al., 2009; Liu et al., 2012a,b), the molecular prevalence of SaV in suckling piglets has never been studied in Hunan province, China. Therefore, we conducted the study on the prevalence of SaV in suckling piglets in Hunan province, China.

In the present study, 45 of the 300 (15%) fecal samples were positive for SaV. The overall molecular prevalence of SaV was far higher than that of other provinces in China (Shen et al., 2009). This may not be surprising because the prevalence of SaV was higher in suckling piglets than in other age groups of pigs (Wang et al., 2006). The overall molecular prevalence of SaV was lower than that found in other countries (Cunha et al., 2010; Reuter et al., 2010); this is most likely due to difference in animal management practices, climates and animal husbandry practices. In the current study, the molecular prevalence of SaV detected (15%) in suckling piglets indicates that 85% of the diarrhea in piglets is caused by some other factors, including bacterial, viral pathogens as well as nutritional and environmental situation. Thus, it is necessary to carry out more testing to further clarify whether or not SaVs are the important cause of diarrhea in piglets.

The phylogenetic relationship of SaV isolates indicates that all the SaV isolates in Hunan province represent SaV/GIII, and they are clearly separated from other SaV reference genogroups (Fig 1). The present results showed genetic variability in the SaV/GIII, which are the most frequent porcine genogroup worldwide (Reuter et al., 2010). Within SaV/GIII, several major clades were present, supporting the existence of extensive genetic diversity in SaV/GIII, such as SaV/GIII-I, SaV/GIII-II and SaV/GIII-III (Dufkova et al., 2013). There is no evidence that pigs are considered as the major reservoirs for human SaV infection; the distinctive phylogenetic locations of the porcine SaV were observed by the present study and also a previous study (Martínez et al., 2006). Interestingly, the present study and previous studies (Shen et al., 2009; Liu et al., 2012a) indicate that SaV/GIII is prevalent in pig population and that SaV/GVI, SaV/GVII and SaV/GVIII are not found in pigs in China, although some studies clearly showed that SaV/GVI, SaV/GVII and SaV/GVIII were detected in pigs in other countries (Martella et al., 2008; Reuter et al., 2010; Yin et al., 2006). In conclusion, the results of the present survey indicate that SaV infections are highly prevalent in symptomatic suckling piglets in Hunan province, but this situation has received little attention in the past. In addition, 85% of the diarrhea in piglets is caused by some other factors, including bacterial, viral pathogens as well as nutritional and environmental situation. Therefore, it is imperative to take integrated control strategies and measures to prevent and control SaV infection in pigs in this province and elsewhere.

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บทคัดย่อ

ความชุกของแซพโพไวรัสในลูกสุกรดูดนมในมณฑลหูหนานประเทศจีน

Sheng-Guo Tan[1] *Na-Liu*[1] *Ping-Yuan Xu*[1*]

แซพโพไวรัสเป็นจุลินทรีย์ก่อโรคในทางเดินอาหารซึ่งเป็นโรคติดต่ออุบัติใหม่ ทำให้เกิดอาการท้องเสียในคนและสัตว์ การศึกษาครั้งนี้ได้ทำการพิสูจน์ทางพันธุกรรมของแซพโพไวรัสในลูกสุกรดูดนมในมณฑลหูหนานประเทศจีนระหว่างเดือนพฤษภาคม 2556 ถึงเดือนพฤษภาคม 2557 โดยนำตัวอย่างอุจจาระที่เก็บมาจากลูกสุกรดูดนมที่มีอาการท้องเสียในมณฑลหูหนานประเทศจีนมาวิเคราะห์ด้วยปฏิกิริยาลูกโซ่พอลิเมอเรสแบบย้อนกลับตามด้วยการหาลำดับเบส ซึ่งตรวจพบแซพโพไวรัสใน 45 จากทั้งหมด 300 ตัวอย่าง (15%) นอกจากนี้การวิเคราะห์แบบแผนวิวัฒนาการแสดงว่าแซพโพไวรัสทั้งหมดถูกจัดอยู่ในแซพโพไวรัสจีโนกรุ๊ป 3 (GIII) การศึกษาครั้งนี้จึงมีผลต่อการควบคุมการติดเชื้อแซพโพไวรัสในสุกรในมณฑลหูหนานประเทศจีน

คำสำคัญ: ประเทศจีน สุกร การวิเคราะห์แบบแผนวิวัฒนาการ แซพโพไวรัสในสุกร ความชุก

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