Genetic evolution and epidemiological analysis of Seneca Valley virus (SVV) in China

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Abstract

Seneca Valley virus (SVV) is a novel Picornaviridae that is closely associated with porcine idiopathic vesicular disease (PIVD). Here, we report the detection and isolation of a novel strain of SVV, CH-GX-01-2019, from swine in Guangxi Province, China. The complete genomic sequence of CH-GX-01-2019 exhibited 93.3 - 98.9% identify with other SVV isolates at the nucleotide level. This new strain of SVV showed the highest level of similarity (98.9%) with Vietnamese strains and exhibited two consecutive mutations in the VP1 gene. Phylogenetic analysis based on the complete genome and the VP1 gene showed that Chinese forms of SVV can be divided into three clusters. We analyzed the geographical distributions of SVV strains in China and found that the epidemiology of these viruses in China is complicated; most strains are distributed predominantly in south and central China. Between 2015 and 2019, the dominant epidemic strains of Chinese SSV changed from clusters 1 and 3 to cluster 2. CH-GX-01-2019 (cluster 3) represents a recombinant strain from Colombia-2016 (cluster 2) and HB-CH-2016 (cluster 1). Our findings will enhance our understanding of the prevalence and genetic variation of SVV in the swine herds of China and provide important insights into the molecular epidemiology of SVV.

1. BACKGROUND

Seneca Valley virus (SVV) is a causative agent of vesicular disease (VD) in swine and belongs to the genus *Senecavirus* within the family of *Picornaviridae* (International Committee on Taxonomy of Viruses, 2017). SVV is a non-enveloped, single-stranded, positive-sense RNA virus (Maggioli et al., 2018). The genome of SVV is approximately 7.3 kb in length and includes a 5'-untranslated region (UTR), a 3'-UTR, and an open reading frame (ORF) encoding the lead protein, four structural proteins (VP1, VP2, VP3 and VP4), seven non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C and 3D), thus forming the standard 'L-4-3-4' structural layout that is typical of the *Picornaviridae* family (Hales et al., 2008, Leme et al., 2017). SVV was first reported as a cell contaminant in 2002 and was named after SVV-001 (Hales et al., 2008). SVV-001 was used as a drug for the treatment of cancer because it exhibits a strong cytotoxic effect on small-cell lung cancer and other neurosecretory tumors (Reddy et al., 2007, Schenk et al., 2020). From a clinical perspective, it is very difficult to differentiate cases of swine infected with SVV from other causative agents of porcine idiopathic vesicular disease (PIVD), such as food-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus (VESV) and vesicular stomatitis virus (VSV) (Leme et al., 2017).

The infection of swine with SVV was first confirmed in Canada in 2007 (Pasma et al., 2008, Leme et al.,

2017). Since then, porcine SSV cases have also been detected in America, China, Brazil, Columbia, Thailand and Vietnam (Leme et al., 2016, Sun et al., 2017, Saeng-Chuto et al., 2018a, Arzt et al., 2019). In China, SVV was first reported in Guangdong province in 2015; subsequently, SVV was detected in populations of swine in Hubei, Fujian, Henan, Heilongjiang, Guangxi Province and Shandong Province (Wu et al., 2016, Wu et al., 2017, Chen et al., 2018, Liu et al., 2019, Bai et al., 2020)). In 2018, the China Animal Health and Epidemiology Center performed a retrospective surveillance study for SVV between 2016 and 2018; the proportion of swine infected with SVV in 2016, 2017, and 2018 were 14.6% (7/48), 21.9% (7/32) and 22.6% (19/84), respectively (Zhang et al., 2019). The gene sequences for these viruses can be obtained from GenBank and can be classified into three clades; clade I is the first identified strain of SVV virus, including SVV-001, clade II is SVV strains of US identified from 1988 to 1997, and clade III contains SVV strains from Brazil, Canada, China, Thailand and the United States from 2001 to 2019; and most of the sequences are concentrated in clade III (Leme et al., 2017). The sequences of SVV in clade III can be clustered into four groups according to the country of origin (Xu et al., 2017). In this manuscript, we report a new strain of SVV that was isolated from Guangxi Province in China and describe the genetic characteristics of this new strain.

2. MATERIALS AND METHODS

2.1 Virus detection, identification, isolation, and growth kinetics

Vesicular fluids and tissue samples were collected from a pig farm in Guangxi Province in the southwest of China. Total RNA was extracted from each sample using TRIzol reagent in accordance with the manufacturer's instructions (Sangon, China). Next, we synthesized cDNA from the total RNA using random primers (primer 9) and M-MLV reverse transcriptase (Takara, China), as described by the manufacturer's guidelines. We also designed a range of specific primers to amplify different viruses (FMDV, SVDV, VESV, VSV and SVV); these primers were designed by Primer Premier 5 (Table S1). Using baby hamster kidney (BHK-21) cell line, SVV was isolated as described previously (Saeng-Chuto et al., 2018b).

BHK-21 cells were seeded into 6-well plates and infected with SVV at a multiplicity of infection (MOI) of 0.1. The cells were then cultured until 14 hours post-infection (HPI). Cells were then fixed with 4% polyformaldehyde (Beyotime) for 20 min, permeabilized with 0.1% Triton X-100 for 7 min, and blocked with 5% skimmed milk (diluted in PBST) for 2 hours. The cells were then incubated for 2 h with a primary mouse-derived anti-VP1 polyclonal antibody (1:500; synthesized in-house). Next, the cells were washed three times in PBST and then incubated with a FITC-labeled goat anti-mouse IgG (H+L) secondary antibody (1:2000) for 30 min. Finally, cells were washed five times in PBST and analyzed under a fluorescent microscope.

In a second experiment, we seeded BHK-21 cells into 6-well plates and infected the cells with SVV (0.1 MOI). Cell culture supernatants were then harvested at 6, 12, 24, 36, 48, and 60 HPI. We then measured the titer of virus in each sample of supernatant by titration. Results are reported as 50% tissue culture infective doses per milliliter (TCID50/ml), in accordance with the Reed-Muench method (Chen et al., 2016). Data were analyzed by GraphPad Prism (version 6.0) software (GraphPad Software Inc., La Jolla, CA).

2.2 Full-length genome sequencing

In order to sequence the full length of the SVV genome, we divided the full sequence into seven segments; primers were designed according to CH-01-2015 (Accession No. KT321458.1) (Table 1). The 5'-UTR and the 3'-poly(A) tail of SVV were sequenced by rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR) (Sangon, China). All PCR products were purified with a Gel Extraction Kit and cloned into the pEASY-Blunt Simple vector (Transgene, China) in accordance with the manufacturer's instructions. The resulting recombinant plasmid was then sequenced (Comate, China).

2.3 Alignment, phylogenetic analysis, and the epidemiology of SVV in China

In total, we downloaded 99 complete SVV genomes from GenBank (Table 2) that were collected between 2002 and 2019 from seven different countries. Next, alignment and phylogenetic analysis was used to analyze the genomes of the isolated SVV; these analyses were based on the complete sequences of SVV and VP1.

Analysis involved the P-distance-based neighbour-joining method in MEGA 7.0 software (Tamura et al., 2011). As VP1 is the main structural protein of SVV and may contain multiple antigen epitopes, we paid particular attention to the possibility of mutation in the amino acids of SVV. MegAlign software (DNASTAR.Lasergene.v7.1) was used to analyze mutations of the amino acids in VP1 of CH-GX-01-2019 and compare these with other isolates. The similarity of the CH-GX-01-2019 genome with other isolates was analyzed by MegAlign software (DNASTAR.Lasergene.v7.1).

In total, 53 SVV isolates in China were collected from GenBank (Table 2). We then analyzed the major genotypes of the strains involved. Then, we generated a genetic tree for the evolution of SVV evolution and then investigated the geographical distribution of the virus.

2.4 Recombination analysis

Recombination event analysis was carried out by analyzing the complete genome for the potential recombination of CH-GX-01-2019. RDP4 (http://web.cbio.uct.ac.za/ ˜darren/rdp.html) (Martin et al., 2010) software (RDP, Bootscan, MaxChi, GENECONV, Chimaera, SiScan and 3Seq), along with SimPlot (http://sray.med.som.jhmi.edu/RaySoft) (Lole et al., 1999) software, were used to analyze events relating to recombination. Recombination signals and the sequences of recombination parental lineages were analyzed using SimPlot.

3. RESULTS

3.1 Virus detection, identification, isolation and growth kinetics

VESV, VSV, SVDV, FMDV and SVV were detected by reverse transcription polymerase chain reaction (RT-PCR). During our analysis, we identified a product of approximately 850bp that was cloned by RT-PCR **(Fig. S1)** . This amplicon was sequenced and confirmed to be part of the nucleic acid sequence of SVV. Virus isolation was performed in a BHK-21 cell line; cytopathic effect (CPE) was observed in the third passage**(Fig. S2)** . Indirect fluorescence assay (IFA) results showed that when treated with anti-SVV VP1-mouse serum, infected cells showed obvious green fluorescence **(Fig. 1A)** . One-step growth curves were obtained after infecting BHK-21 cells with CH-GX-01-2019; analysis showed that the maximal viral titers of CH-GX-01-2019 were approximately $10^{7.0}$ $TCID_{50}$/ml **(Fig. 1B)** .

3.2 Genome analysis of SVV in China

Next, we sequenced the complete genome of the isolated SVV strain (CH-GX-01-2019: Accession No. MT457474). Our analysis revealed a 7303 bp sequence consisting of a long 5'-UTR of 668 bp, an ORF encoding a 2,181 amino acid polypeptide and a short 3'-UTR of 89 bp (Table S2).

We compared the similarity of the complete genomic nucleotide sequence of CH-GX-01-2019 with other isolates from across the globe. The similarities between CH-GX-01-2019 and 99 other complete sequences of SVV ranged from 93.3 - 98.9% **(Table S3).**

3.3 Phylogenetic analysis of SVV in China

Genetic analysis, based on the complete sequences of SVV, revealed that the Chinese isolates of SVV were mainly grouped into three clusters (clusters 1-3), thus indicating the complexity and sequence diversity of these strains. CH-GX-01-2019, was classified into cluster 3 and belonged to one branch of the CH/GXI09/2016 strain (KY038016.1). CH-GX-01-2019 shared 96.7% nucleotide identity to the first reported strain in China (CH-01-2015; KT321458). The homologies of the nucleotide sequence for CH-GX-01-2019 with cluster 1, cluster 2 and cluster 3 of the Chinese strains were 96.0 - 96.5%, 96.1 - 96.8% and 98.2 - 98.7%, respectively **(Fig. 2A)** . Surprisingly, CH-GX-01-2019 showed the highest similarity (98.9%) with the 2018 Vietnam strain (MH704432.1).

With regards to the VP1 sequence of SVV, the Chinese isolates of SVV were mainly grouped into three genetic clusters (clusters 1 - 3). The homologies of CH-GX-01-2019 with cluster 1, cluster 2 and cluster 3 of the Chinese strains were 95.3 - 95.7%, 94.2 - 96.1% and 97.7 - 98.5%, respectively. Variations in the VP1

amino acid sequence were generally observed at codons 230 (T/I) and 231 (A/S); these changes were critical to the variations observed between the different viruses **(Fig. 2B)** .

3.4 Epidemiological analysis of SVV in China

SVV isolates from China showed a range of different features; some strains were closely related with isolates from the USA (cluster 2), while some strains were more related to those from Brazil (cluster 3) or Canada (cluster 1). In total, 54 SVV isolates (including CH-GX-01-2019) were collected from different regions of China, including Guangdong (33/54), Hubei (2/54), Fujian (2/54), Henan (7/54), Heilongjiang (1/54), Guangxi (3/54), Shandong (1/54), Anhui (2/54), and Sichuan (1/54) Province; two isolates came from unknown regions. Further analysis showed that Chinese isolates predominantly belonged to cluster 1 and cluster 3 between 2015 and 2016, while those from 2017 belonged to cluster 2 **(Fig. 3A)** .

Genetic analysis, based on the complete sequences of SVV revealed that SVV isolates from Guangdong Province were distributed in three clusters and that 33 sequences shared 95.9 - 99.9% identity with each other. Isolates from Hubei, Fujian, Henan, Heilongjiang, Shandong Anhui and Sichuan Province were classified into cluster 2, and were similar to isolated from the USA. Some isolates from Henan, Guangxi, and Guangdong Province, were classified into cluster 3, and were similar to isolates from Brazil. Other isolates, from Hubei and Guangdong Province, were classified into cluster 1, and were similar to isolates from Canada. Further studies showed that SVV was detected in Liaoning, Shanghai, Hainan, Guizhou, Yunnan, Sichuan, Gansu and Xinjiang Province in China. Analysis shows that the epidemiology of SVV in China is complicated, although most isolates are found predominantly in southern and central regions of China **(Fig. 3B).**

3.5 Recombination analysis

RDP4 software was used to analyze recombination events. Analysis showed that Colombia-2016 (KX857728.1) could be a potential major parent while HB-CH-2016 (KX377924.1) could be a potential minor parent. Our results provided strong statistical support for a recombination event involving CH-GX-01-2019 (P <0.01). SimPlot analysis revealed the existence of breakpoints that separated the genome of CH-GX-01-2019 into two fragments; one fragment originated from Colombia-2016 (1-1903 nt) while the another fragment originated from HB-CH-2016 (1903-7303 nt)**(Fig. 4)** .

4. DISCUSSION

The first strain of SVV was detected from swine in Canada in 2007 (Pasma et al., 2008); since then, several SVV-infected swine outbreaks have been reported. Since 2015, many countries have reported SVV epidemics or small-scale outbreaks (Leme et al., 2019). As a new infectious disease, SVV may result in huge economic losses. The clinical symptoms of SVV in swine are fever, lethargy, and anorexia; in addition, blisters on the skin or mucous membranes of the nose, mouth, tongue and hoof, are common. It is difficult to distinguish between SVV and VESV, VSV, SVDV, and FMDV according to clinical symptoms (Zhang et al., 2018). Studies have shown that SVV can be detected in infected swine for a period of 7-21 days; the clinical symptoms last 12-14 days and viremia can last 1-10 days (Maggioli et al., 2018). In addition, SVV has been detected in the intestines and feces of mice, and also in house flies, thus implying that these species may carry pathogens for propagation (Joshi et al., 2016).

In a previois study, Chinese isolates of SVV were divided into five clusters (Wang et al., 2019). Previous genetic analysis indicated that SVV isolates from the USA, Brazil, and Canada, were all clustered onto one branch of an evolutionary tree and that Chinese isolates spread out over one branch with isolates from the US, Brazil, or Canada (Xu et al., 2017, Chen et al., 2018). The genetic analysis carried out in the present study indicated that the SVV isolates from China can be divided into 3 clusters. Based on this, we can infer that the origins of the SVV strains currently circulating in China may be diverse. We also analyzed the genetic evolution of the Chinese strains in order to provide a foundation with which to prevent and control SVV in the future.

The first infection caused by SVV was reported in the Guangdong province of China in 2015 (Wu et al., 2017, Wu et al., 2016). Since then, cases of SVV infection have been detected and reported in several

provinces of China (Zhang et al., 2019). Therefore, it is vital that we analyze the geographical distribution of different viral subtypes. So far, most Chinese isolates of SVV isolates are distributed in the south and central regions of China. Furthermore, our results indicate that different SVV genotypes co-exist in China. Furthermore, our results showed that the classification of the Chinese isolates have changed from clusters 1 and 3 to cluster 2. In China, the swine population is very dense and highly mobile; collectively, these factors have contributed to the diversity of the Chinese SVV isolates (Guo et al., 2020). These data provide further understanding with regards to the path of SVV propagation in China.

Four cases of SVV recombination events have been reported in China: HeN-1/2018 (960-2354 bp), HB-CH-2016 (position 1563 bp), SVA/CHN/10/2017 (4145-5620 bp) and HeNNY-1/2018 (4190-5808 bp). These recombination events predominantly occurred in the P1, P2, and P3 regions. Combined with our data, these results showed that the current recombinant regions are concentrated in the P1, P2 and P3 regions; however, the current dataset is limited and cannot fully represent the full extent of recombination in the SVV strain. The recombination events involving Chinese SVV isolates indicate that SVV recombination events may have occurred as early as 2016 (Wang et al., 2018, Guo et al., 2020). CH-GX-01-2019 (Cluster 3) is a strain that represents a recombination of Colombia-2016 (Cluster 2) and HB-CH-2016 (Cluster 1). It is possible that different clusters of virus strains could recombine in the pig breeding environment to form new strains. Viral recombination between different clusters may accelerate phenotypic variation, thus allowing the recombinant virus to escape surveillance by the host's immune system and thus survive for longer in the host. Whether the new strain of virus (CH-GX-01-2019) exhibits variation in terms of pathogenicity and antigenicity needs to be ascertained as a matter of urgency. Furthermore, recombination events and the effects of mutation on the final amino acid sequence may lead to diversity and add to the complexity of SVV, thus changing the phenotype and functionality of the virus (Guo et al., 2020).

5. CONCLUSIONS

In conclusion, we detected and isolated a new strain of SVV and carried out analysis related to genetic evolution and geographic distribution. The new isolate showed the highest levels of similarity with the 2018 Vietnam strain (MH704432.1), thus representing a cause for concern with regards to the spread of the virus across borders. Genetic recombination and the effects of mutation on the final amino acid sequence of SVV may affect virulence and key characteristics of the virus and thus increase the adaptability of the virus in pigs. These results provide further insight into the epidemiology of SVV and will allow us to strengthen surveillance for potential SVV virus epidemics in China and develop strategies to prevent the spread of SVV if necessary.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

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

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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HIGHLIGHT

1. A novel SVV strain (CH-GX-01-2019) was isolated from Guangxi, China.

2. Genome analysis of the SVV in China were analyzed in this study.

3. The geographical distributions of the SVV in China were analyzed in this paper.

4. Genetic recombination of CH-GX-01-2019 was confirmed in this study.

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