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Short communication

Isolation and phylogenetic analysis of an emerging Senecavirus A in China, 2017

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ABSTRACT

Senecavirus A (SVA), which is associated with porcine vesicular disease and high mortality in neonatal piglets, is a small non-enveloped RNA virus and a member of *Picornaviridae* family. An emerging SVA strain, named SVA CH/FuJ/2017, was isolated from vesicular liquid and vesicular lesion tissue from piglets with vesicular disease in Fujian province, China. In our study, the complete genome sequence of SVA CH/FuJ/2017 strain has been determined. The viral genome was 7285 nt in length. The homology analysis indicated that the gene sequences of polyprotein and VP1 in SVA CH/FuJ/2017 shared highest nucleotide identities with American SVA isolates; and polyprotein showed the highest similarity with American SVA isolates. The phylogenetic analysis based on polyprotein and VP1 nucleotide sequences indicated that SVA CH/FuJ/2017 was closely related to American SVA isolates. The results revealed that the novel SVA strain was closely related to those SVA strains that were isolated in America. Hence, the retrospective study is important for tracing the probable origin of China SVA strains.

Senecavirus A (SVA), also known as Seneca Valley virus (SVV), is a non-enveloped RNA virus belongs to Senecavirus genus, *Picornaviridae* family (Wu et al., 2016). The SVA genome, is approximately 7.3 kb, a single-stranded and positive-sense RNA, encodes a single polyprotein which is cleaved by viral and cellular proteases to produce mature proteins (Hales et al., 2008). In 2002, SVA was first discovered by Genetic Therapy Inc. (Gaithersburg, MD, USA) in cell culture of human fetal retinoblasts (Hales et al., 2008). Originally, SVA was not associated with any specific pathology and a lot of studies focused on its oncolytic activity in cancer therapy (Hales et al., 2008; Burke, 2016). However, the evidences that SVA was associated with porcine idiopathic vesicular disease (PIVD) were confirmed in Canada in 2008 and USA in 2012, respectively (Pasma et al., 2008; Singh et al., 2012). The clinical signs caused by SVA, Foot and Mouth disease virus (FMDV), Swine Vesicular Disease Virus (SVDV), Vesicular Stomatitis Virus (VSV) and Vesicular Exanthema of Swine Virus (VESV) are indistinguishable (Sáiz et al., 2003; Lung et al., 2011; Fernández et al., 2008; Bracht et al., 2016; Leme et al., 2015). Since the end of 2014, an increasing number of SVA outbreaks in different countries have been reported, such as USA, Brazil, Canada, China, Colombia and Thailand (Guo et al., 2016; Qian et al., 2016; Saengchuto et al., 2017; Saporiti et al., 2017; Sun et al., 2017).

In China, the first SVA isolate was identified from sows with vesicular lesions and acutely dead neonatal piglets in Guangdong, China (Wu et al., 2016). After that, more and more SVA isolates were identified from other provinces in China (Wang et al., 2017; Wu et al., 2016; Zhao et al., 2017; Zhu et al., 2017). Among different cases, similar clinical symptoms of affected swine were observed (Wu et al., 2016; Zhu et al., 2017; Zhao et al., 2017). Here, we reported a SVA infection and the complete genome of SVA CH/FuJ/2017.

In July 2017, vesicular disease broke out in a swine herd in which all pigs were compulsorily vaccinated with FMDV vaccine in Zhangzhou, Fujian province, China. Fluid-filled vesicles and ulcerative lesions were discovered on the foot and snout on piglets and growing-finishing pigs (Fig. 1). In the herd, some pigs were lame due to ulcerative lesions. A total of 10 samples, including 5 vesicle fluid samples and 5 vesicular lesion tissue samples, were collected from the pigs presenting vesicular disease.

The total RNA of all samples, template for the detection of viral pathogens that cause vesicular disease, were extracted using Viral RNA Extraction Kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) according to the manufacturer's instructions. RT-PCR was performed to detect the pathogen of vesicular disease using their specific primers (Table 1). Virus was isolated form SVA-positive samples using BHK-21

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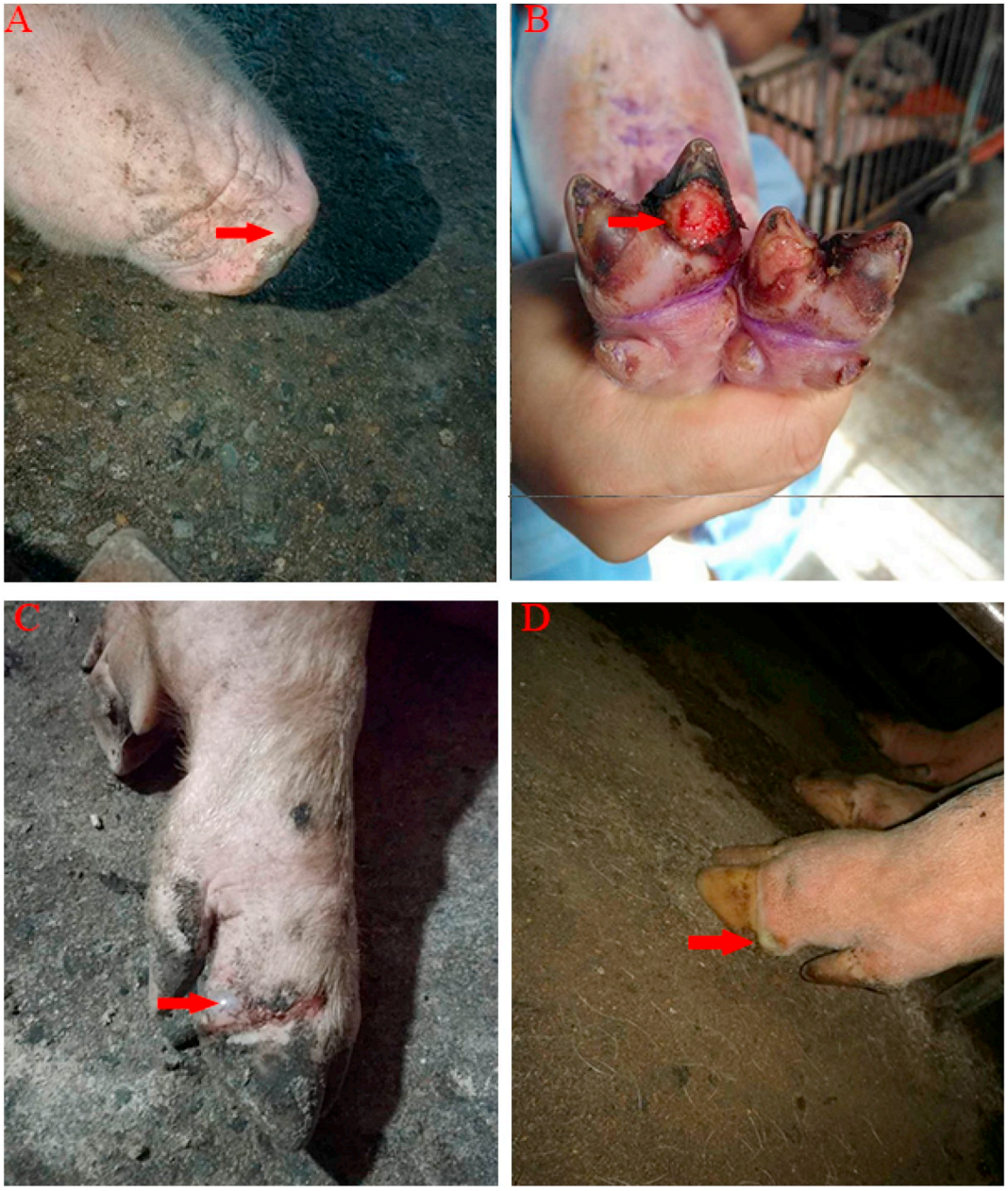

Fig. 1. Clinical signs of SVA infection. Fluid-filled vesicles on the snout (A) and the coronary band of the pigs (C, D), and rupture lesions on hooves of the pigs (B). The red arrow showed lesions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Specific primers using to detect vesicular disease-associated pathogens.

Primer Name	Sequence(5'to3')	Fragment Length	Reference
SVV-F	GCTGTAAAAACCTTCTC	326 bp	Wu et al., 2016
SVV-R	ATAGTATGTGCCAAGAG		
FMDV-F	CACACGGCGTTCA CCCAWCGC	290 bp	Sáiz et al., 2003
FMDV-R	GACAAA GGTTTTGTTCTTGGTC		
SVDV-F	GGCCAACTTTGAGGAGGCAATC	207 bp	Núñez et al., 1998.
SVDV-R	TAAGGGTAACCTGCACTGGTGG		
VSEV-F	CGACTCGATGGACCTGTTCACATACG	694 bp	Lung et al., 2011.
VSEV-R	CGTAGAGGTCGGTTAGGTCCTTTCTG		
VSV-F	AATGACGATGAGACYATGCAATC	110 bp	Fernández et al., 2008.
VSV-R	CAAGTCACYCGTGACCATCT		

cells, PK-15 cells, and ST cells. The complete genome of the SVA isolate was amplified using specific primers as described previously by RT-PCR (Wu et al., 2016). The products of RT-PCR were purified and cloned into pMD18-T (TaKaRa Biotechnology (Dalian) Co., Ltd.). The recombination clone vectors were sequenced by BioSune Biotech Co. Ltd. (Beijing, China). The complete genome was assembled with Seqman software in DNAStar (DNASTAR, Madison, WI, USA). The reference SVA complete genome sequences were selected from GenBank for phylogenetic analysis. The phylogenetic tree was constructed by MEGA 6.0 software using maximum likelihood method under the best model with 1000 bootstrap replicates.

All the samples collected from the infected pigs from the swine farm in Zhangzhou, FuJian province were SVA positive whereas other vesicular disease-associated pathogens (FMDV, SVDV, VSV and VESV)

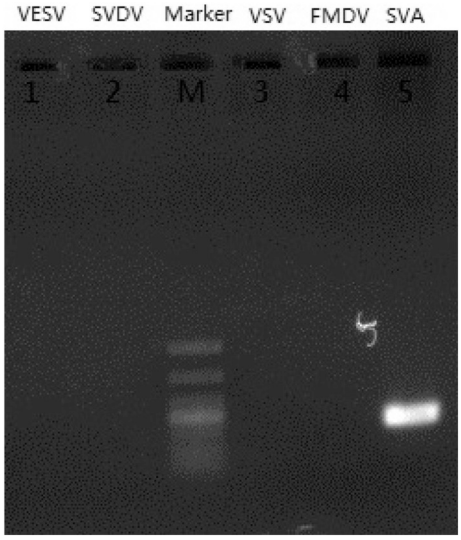

Fig. 2. Detection of vesicular disease-associated pathogens by RT-PCR. Lane 1 is for VESV; Lane 4 is for FMDV; Lane 2 is for SVDV; Lane 3 is for VSV; Lane 4 is for FMDV; Lane 5 is for SVA; Lane M is DNA marker (from top to bottom is 1000–700–500-400-300-200-100 bp).

showed negative (Fig. 2). We successfully isolated a novel SVA strain, named SVA CH/FuJ/2017. BHK-21, PK-15 and ST cells challenged with SVA CH/FuJ/2017 showed typical cytopathic effects (CPE) which were characterized as rounding, shrinkage and fragmentation (Fig. 3). The viral tissue culture infective dose 50 ($TCID_{50}$) of the third culture in BHK-21 cells was $10^{-8.2}$/0.1 ml. The $TCID_{50}$ in ST cells and PK-15 cells were up to $10^{-7.0}$/0.1 ml and $10^{-6.67}$/0.1 ml, respectively.

The complete genome of the isolate was sequenced and analyzed. The complete genome sequence of SVA CH/FuJ/2017 was submitted to GenBank and the assigned GenBank accession number is MH490944. The open-reading frame (ORF) sequence of SVA CH/FuJ/2017 shared 93.7%–98.5% nucleotides identity with other reference sequences, and showed the highest identity with USA/GBI29/2015 strain which was isolated in 2015 in United States of America (USA) (GenBank accession No. KT827251) as well as HB-CH-2016 strain which was isolated in 2016 in China (GenBank accession No. KX377924). As for amino acids comparison, SVA CH/FuJ/2017 shared 99.3% similarity with the isolates including USA/IA40380/2015, US-15-40380IA, US-15-40381IA that were isolated in America (GenBank accession No. KT757280, KU051392, KU051393, respectively). However, SVA CH/FuJ/2017 showed 99.2% similarity with HB-CH-2016, and 98.3%–99.1% similarity with other isolates previously reported in China. Forty-nine SVA ORF sequences obtained from GenBank along with the ORF sequence isolated in our study were used to construct the phylogenetic tree by MEGA 6.0 software. The phylogenetic tree indicated that the SVA CH/FuJ/2017 was closely related to the American strain USA/GBI29/2015, however, it was distantly related to other China strains that were isolated previously (Fig. 4).

Capsid protein (VP1) is the main epitope region which is able to induce SVA neutralizing antibody. VP1 is also associated with SVA cell tropism. Thus, it is necessary for us to know the genetic evolutionary relationship based on VP1 sequence. In our study, SVA CH/FuJ/2017 and sixty-four reference SVA sequences were used for comparison analysis. The nucleotide sequence of VP1 gene of CH/FuJ/2017 presented 95.1%–97.6% nucleotide identity with other Chinese SVA isolates while it shared higher nucleotide identity with American isolate KS15–01(KX109804). Nevertheless, it just shared 86.7%–92.8% similarity with American earlier SVA strains (GenBank accession No. EU271757, EU271758, EU271759, EU271760, EU271761, EU271762, and EU271763). For amino acid comparison, SVA CH/FuJ/2017 showed 98.5%–99.6% similarity with other Chinese isolates. However, it showed higher similarity with American isolates as well as the Chinese SVA/HLJ/CHA/2016 strain (GenBank accession No. KY419132). These results are consistent with the nucleotide identity. The phylogenetic tree constructed based on sixty-five VP1 sequences indicated that SVA CH/FuJ/2017 was closely related to American isolates isolated in 2015. It also showed that SVA CH/FuJ/2017 strain kept farther distant with branches consisted of Chinese SVA isolates and American earlier SVA strains. From the phylogenetic analysis, American earlier isolates and recent SVA isolates from various countries were found in different branches (Fig. 5).

In the swine farm, all pigs were vaccinated with FMDV vaccine before appearance of vesicular disease. After the animal presented the symptoms of vesicular disease for the first time, other healthy pigs subsequently showed similar clinical symptoms (Fig. 1). This phenomenon was consistent with previous research (Zhu et al., 2017). According to the homology and phylogenetic analysis, SVA CH/FuJ/2017 shared higher nucleotide sequence identity and amino acid similarity with American SVA strains and was closely related to American SVA strains. Phylogenetic trees based on SVA ORF sequences and VP1 gene sequences provided evidences that the virus of SVA CH/FuJ/2017 strain in this study might have been introduced into China due to the import of pigs. However, this hypothesis needs further investigation and retrospective research that will give the answer when the virus begins to circulate in pig herds in China.

Previous research, SVA prototype strains were nonpathogenic to pigs while the recent SVA strains isolated since 2015 showed increasing pathogenicity to pigs (Burke, 2016; Hales et al., 2008; Pasma et al., 2008; Singh et al., 2012). The changed pathogenicity of SVA may be caused by the mutation in its genome, and this hypothesis should be further investigated to reveal the genetic and virulence evolution mechanism of SVA.

SVA, an emerging infectious pathogen, is circulating in American swine herds since 1980s (Hales et al., 2008). However, SVA prototype strain-SVV-001 (GenBank Accession NO. DQ641257) was isolated from PER.C6 cell line (Hales et al., 2008). There was no evidence about SVA pathogenicity to pigs until that SVA was associated with swine vesicular disease reported in Canada (2008) and America (2012), respectively (Pasma et al., 2008; Singh et al., 2012). Since 2015, SVA was isolated in many countries including USA, China, Canada, Brazil, Colombia, and Thailand; and the virus was found to be associated with the increasing piglet mortality rate. Until now, more and more SVA strains were isolated and identified, but there are still lack of researches on SVA pathogenicity, propagation characteristics, and the genetic evolution and so on. We can't distinguish SVA from FMDV, SVDV, VSV, and VESV only by the clinical symptoms. Hence, rapid differential diagnosis technique is urgently needed for the prevention and control of vesicular disease in order to reduce the economic loss caused by SVA infection; and more attention should be paid on SVA infection and transmission.

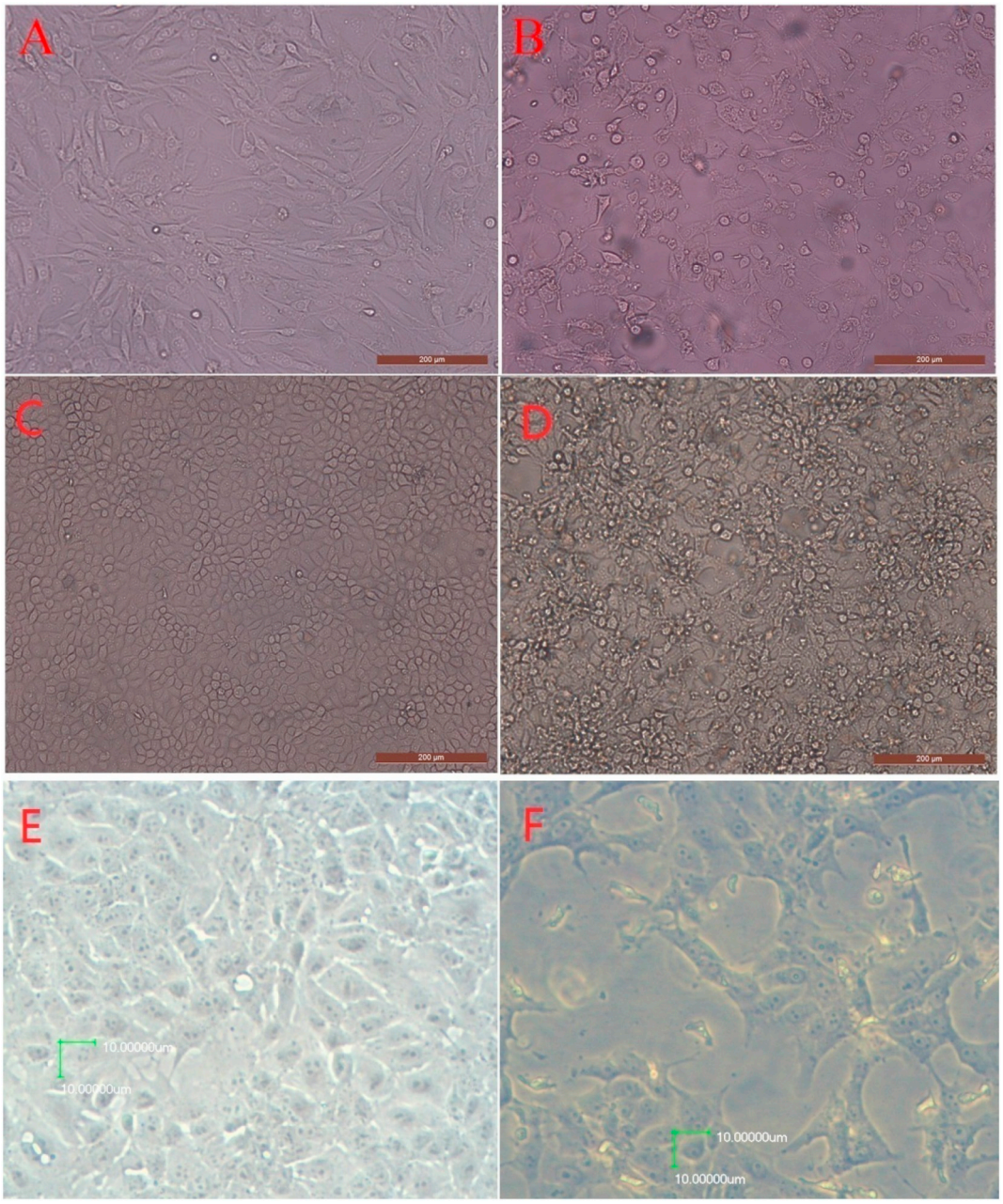

Fig. 3. The cytopathic effects (CPE) on different cell lines infected with SVA CH/FuJ/2017 strain. A: BHK-21 cells uninfected with SVA CH/FuJ/2017 strain; B: BHK-21 cells infected with SVA CH/FuJ/2017 strain; C:PK-15 cells uninfected with SVA CH/FuJ/2017 strain; D: PK-15 cells infected with SVA CH/FuJ/2017 strain; E:ST cells uninfected with SVA CH/FuJ/2017 strain; F: ST cells infected with SVA CH/FuJ/2017 strain.

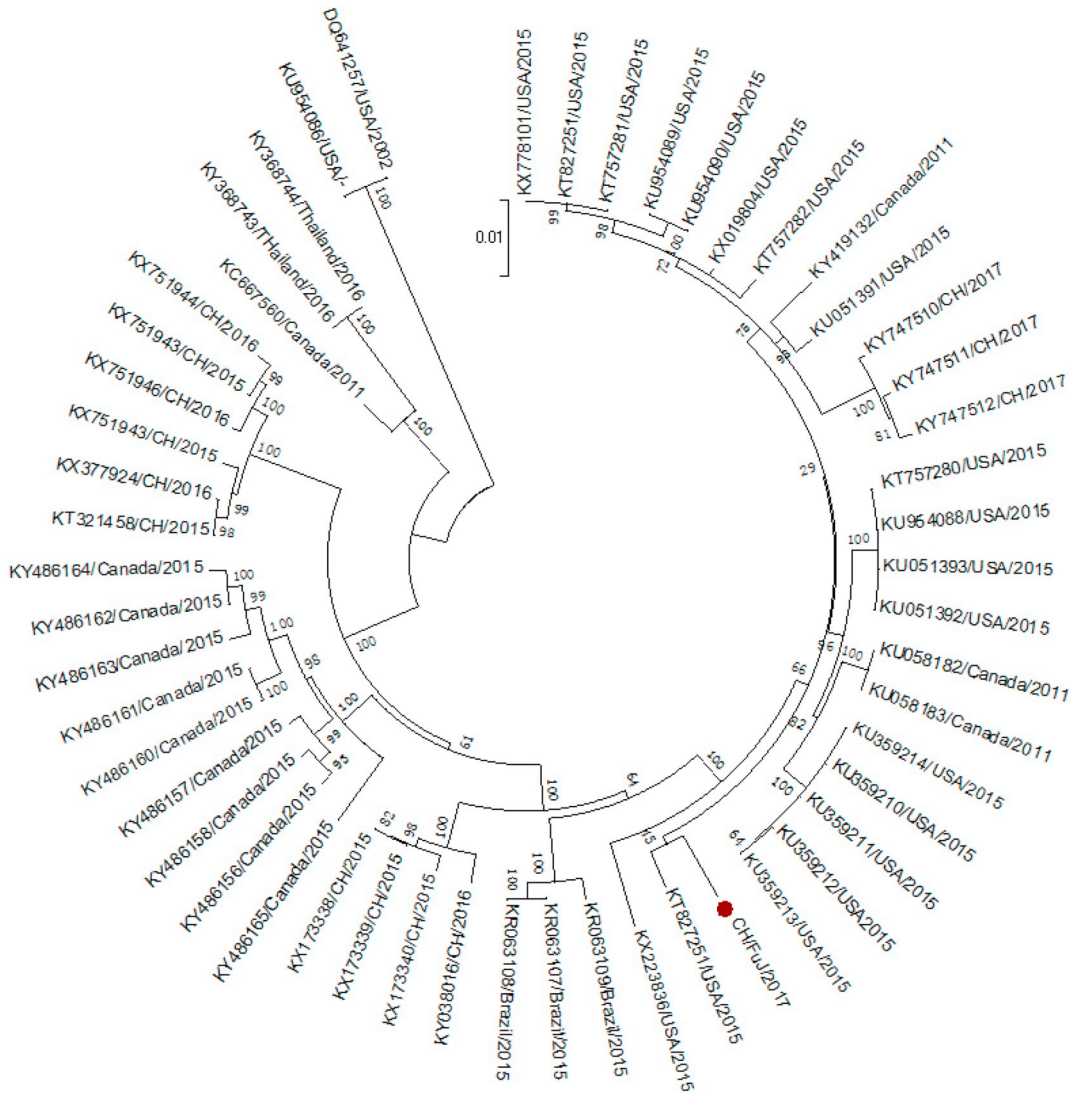

Fig. 4. Phylogenetic analysis of SVA polyprotein nucleotide sequences. Phylogenetic tree was constructed using Maximum likelihood method, with the best model TN93 + G, with 1000 bootstrap replicates, using MEGA6.0 software. The red blot is SVA CH/FUJ/2017. The number on every branches indicates bootstrap values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Phylogenetic analysis of SVA VP1 nucleotide sequences. Phylogenetic tree was constructed using Maximum likelihood method, with the best model TN93 + I, with 1000 bootstrap replicates, using MEGA6.0 software. The red blot is SVA CH/FUJ/2017 isolated in this study. The red box indicated SVA isolates from China. The number on every branches indicates bootstrap values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Conflict of interest statement

The authors declare that they have no competing interests.

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