Isolation and Characterization of Seneca Valley from pig Transboundary spread to the Mink Infection

Ziliang Qin[1], Xinmiao He[2], Chao Chen[1], Zida Nai[1], Yao Wang[1], Di Liu[2], and Xinpeng Jiang[1]

[1]Northeast Agricultural University
[2]Heilongjiang Academy of Agricultural Sciences

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Original article

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Ziliang Qin[1#], Xinmiao He[2#], Chao Chen[1], Shaojun Chen[1], Zida Nai[1], Yao Wang[1], Wentao Wang[2], Gang Li[1], Fang Wang[2], Ming Tian[2], Haijuan He[2], Heshu Chen[2], Di Liu[1,2*], Xinpeng Jiang[1*]

1. Northeast Agricultural University, Harbin, Heilongjiang 150030, P. R. China
2. Key Laboratory of Combining Farming and Animal Husbandry, Ministry of Agriculture, Animal Husbandry Research Institute, Heilongjiang Academy of Agricultural Sciences No. 368 Xuefu Road, Harbin 150086, P. R. China

#The authors contributed equally to this study.
* Corresponding author. Tel.: +86-451-55190722
E-mail address: jiangxinpeng@neau.edu.cn (Xinpeng. Jiang)
liudi1963@163.com

ABSTRACT

Seneca Valley Virus (SVV) infection has recently disseminated across pig farm in Canada, American and China. SVV has been identified in human, rodents, and houseflies. Although cross-species transmission events may lead to limited subsequent transmission, sustained outbreaks have been observed in a new mammalian host. Thus, in our study we utilized molecular characteristics, pathological examination, and immune respond to ascertain whether mink could serve as a novel mammalian host for SVV genomes. Here, our study utilized the porcine strain of SVV to orally infect minks, resulting in pathological changes observed in the intestines. Besides, the SVV could stimulate the specific neutralizing antibody response. The neutralizing antibody of SVV have also been found from mink with an epidemiological investigation in the Heilongjiang province. This study accentuates the role of SVV infection in minks as an impetus for viral evolution, posing potential threats to livestock, public health, and economic prosperity.

Keywords: Seneca Valley Virus, Cross-host transmission, Mink

1.Introduction

The picornaviruses commonly infect a wide range of animals and humans. They cause a broad clinical symptom, such as myocarditis, meningitis, encephalitis, diarrhea and paralysis. Recently, the research has focused on the dependency between the host transmission and the picornaviruses infection in intestines. There were a lot of studies shown that the picornaviruses broke host barriers that shared closer genetic similarities between species. The different viral genera can break their adaptation to new hosts, such as Kobuviruses, Foot-and-mouth disease virus and Enterovirus[1-3]. Seneca Valley Virus (SVV) is a single-stranded positive-sense RNA, belongs to the genus *Senecavirus* within the family *Picornaviridae*, which is closely related to genus *Cardiovirus,* and they are known for infecting vertebrate animals, including pigs, mice and humans. The histopathology revealed that the SVV caused pathological change in epithelial cells and acute lameness myocarditis, such as vesicular lesions, interstitial pneumonia, and atrophy of intestinal villi with vacuolation of the superficial epithelial

cells[4, 5]. It has been demonstrated that the virus is able to spread to other internal organs without any other clinical manifestations.

The concept of 'sentinel species' within the cross-species transmission is important in the public health sciences because sentinel species can provide the best animal model for the further research with the Seneca virus, that provided the integrated and relevant information on the virus evolution through adaptive mutations and neofunctionalization[6, 7]. The mink (Mustela vison), a member of the weasel family, is a carnivorous mammal which occupy a high trophic status in the wild. Its diet consists of a wide variety of animals including mice, frogs, snakes, birds and small mammals. The mink has been found with many virus infections by the research in the cross-species transmission. Recently, humanity is facing a pandemic of a new coronavirus, SARS-CoV-2. There was report SARS-CoV-2 infection of mink on two farms in the Netherlands[8]. Interestingly, mink as a neglected mammalian host, was infected by more subtypes of influenza A viruses, including both mammalian influenza A viruses and avian influenza A viruses[9]. In epidemiological survey, minks, could possibly be an important sentinel species for virus surveillance and early warning. However, there was still many major problems to be studied with the mink for the dynamics of cross-species transmission in the models in relevant settings. More importantly, most studies have relied on prospective inference and reconstruction in the infection, without the mechanism research in the immunity.

Here, the SVV in this study was from a farm, which was used in the infection of mink, and describe the associated clinical signs, pathological and virological findings. Sequence analysis of SVV implied the role as a probable source of the initial infection, point at transmission between mink, mice and pig, which was also hazardously excreted by mink in the environment for the persistent infection in the wild environment. This study is the first detection of the cross-species transmission with SVV in the mink.

2. Material and Method

2.1. Ethics statement

This study was abided to the animal welfare guidelines of the World Organization for Animal Health. All of the clinical animal samples used in this study were approved by the Committee on ethics from the animal science and technology collage of Northeast Agricultural University for routine testing. The animal health code was NEAU201918.

2.2The cell reagents and the virus isolation

Baby hamster kidney 21 (BHK-21) cells cultured in Dulbecco's muddied Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) were grown at 37℃in a humidified 5% CO2 incubator. The BHK21 cell line was utilized for the proliferation of SVV, using tissue samples obtained from infected piglets on a farm in Heilongjiang Province, from which our laboratory isolated the SVV strain found in the vesicular sample collected from the infected piglets. The SVV viral RNA was extracted from the vesicular sample, and which was converted into cDNA using reverse transcriptase HiScript®Q RT SuperMix (+gDNA wiper) and synthetic cDNA primers (Vazyme, Nanjing, China). The cDNA as a follow-up template, PCR analysis of SVV-specific primers. The purified PCR products was sequenced (Genscript, Nanjing, China). The primer of SVV-1/2 were used to amplify the VP1 of SVV, and the other vesicular disease viruses, such as FMDV (serotypes Asia 1, O, and A), VSV, SVDV, and VESV were amplify using RT-PCR. The method of virus isolation was used with BHK-21 cells as previous report. The infectious sample were harvested for 2 days until the cytopathic effect (CPE) was observed[10] (Figure1A). The harvested CPE was cultured in the BHK-21 cell for examination of SVV with RT–PCR as described above. The isolated strain was named SVV-CH-09-2018. The SVV-CH-09-2018 was added to BHK-21 cellscells at an MOI of 0.5 and 1 for one-step growth curves [11](Figure1B).

2.3 Cell preparation for Transmission electron microscopy

The stained SVV-CH-09-2018 was observed with a transmission electron microscope (TEM). Viruses of SVV-CH-09-2018 infected the BHK-21 cells for 18 hours, which were washed with pre-cooled PBS twice, and fixed with glutaraldehyde at 4 ℃. The scraped cell was centrifuged at 2000g for 10min, and the supernatant was discarded. The sample was used to post-fix in the OsO4, which were embedded in epoxy resin, and polymerized at 80 ℃ at least for 3 days. At last, the cells sample were cut into 60-

nm slices for staining with uranyl acetate [12] .The grids were observed under TEM (Hitachi HT7600 TEM, Japan).

2.4 Sequencing and phylogenetic analyses

The SVV-CH-09-2018 whole genome is divided into seven overlapping fragments. Seven fragments were PCR amplified using Prime STAR®HS (TaKaRa, Dalian, China). Primers are shown in Table 1. The PCR product was purified and cloned into pEASY®-Blunt Simple clone vector (Transgene), and the cloned products were sequenced by Sangon Biotech (Shanghai, China). Using the SeqMan II program in the DNASTAR software package (DNASTAR, Madison, WI, USA) to assemble seven overlapping fragment sequences into a complete genomic sequence, using 5'-full RACE core kit and 3'-full RACE core kit (TaKaRa, The specific primers of Dalian, China) were used to detect 5'- and 3'-utr (Table 1), and the sequences targeted by the primers were designed by the existing SVV sequence with the PCR product sequence.

In the construction of gene development tree we first analyzed the SVV-CH-09-2018 gene sequence by BLAST. After obtaining and filtering similar sequences, we screened Seneca genes from several countries, including the United States, China, Canada, Vietnam, Colombia, and Brazil. we use MEGA7.0 and OMICSTUDIO evolutionary tree software for phylogenetic genome analysis, After aligning selected block by clustal, using the Neighbor-Joining method , In the phylogeny test, we used the Bootstrap method and the amount Of Bootstrap replications was set to 1000. Evolutionary tree processing is continued in OMICStudio after the preliminary evolutionary tree is obtained.

2.5 Mink challenge assay

The SVV-CH-09-2018 strain was used for the challenge test. Choose minks that have been weaned for about 60 days(half male and half female) to observe for 1 week to ensure asymptomatic. SVV, FMDV, SVDV, VSV, and pseudorabies virus were not detected by the corresponding ELISA antibody kit and RT-PCR or PCR methods. Two minks are divided into two groups. The first group was injected intraperitoneally with strain SVV-CH-09-2018 for 5 mL (1×10^{9}TCID50/mL), and the second group was inoculated with DMEM as a negative control (NC). Both groups of minks were fed under the same conditions and in separate rooms, where strict biosafety protocols were followed to avoid crossover. contamination their clinical symptoms are monitored daily

for 28 days. At 0, 3, 7, 14, 21, or 28 days after the challenge (d.p.c.), mink serum was collected and the anti-SVV neutralizing antibody titer was determined[13]. TaqMan real-time RT-PCR was used to detect viral load[14] [15]. A standard curve was generated by plotting the threshold values against the serially diluted plasmid DNA encoding the SVV VP1 gene fragment. At 28 d.p.c. the mink were euthanized for the pathological examination. The heart, spleen, liver, kidney, lung, inguinal lymph nodes and other organs were taken for histopathological observation. As mentioned previously, TaqMan real-time RT-PCR was used to detect the mRNA of virus titer in these organs.

2.6 Pathological and Immunofluorescence examination

Intestinal tissues were collected for the pathological and immunohistochemical examination within 10 to 15 minutes after the mink died. Intestinal tissue was impregnated with formalin for 4 h and soak in ethanol of different concentration gradients for 2 hours. And the samples were used to embedd in paraffin wax. The paraffin sections with 6 μm were stained with hematoxylin–eosin (H&E) for the histological examination, which used the light microscopy (Olympus, Tokyo, Japan). And the dewaxing sections were used the immunofluorescence assay[16]. The sections were blocked with the 0.3% bovine serum albumin (BSA) in PBS at room temperature (RT) for 3 h, which were washed with pre-cooled PBS for three times. After the permeabilization with 0.4% Triton X-100 at room temperature, the sections were incubated for 45 min at RT with the specific antibody for the anti-SVV of VP1 antibody, which was made by our lab. The second antibody of goat anti-mouse IgG H&L antibody (FITC) (Abcam) were used in the sections for 30 min at RT. Finally, the samples were washed and examined under a fluorescence microscope (Leica, Wetzlar, Germany).

2.7 The production of specific SVV antibody

All minks received the same feeding conditions. Their clinical symptoms are monitored daily for 28 days. At 0, 3, 7, 14, 21, or 28 days after the challenge (d.p.c.), the mink serum was collected for the examining the quality of specific antibody with the ELISA method. The protein of VP1 protein was obtained by prokaryotic expression, which was produced by our lab. The second antibody of horseradish peroxidase-conjugated (HRP) Rabbit Anti-Mink IgG/HRP(SolarBio, Beijing, China) was added, and then incubated

for 1 hour in RT, and then washed three times. The substrate o-phenylenediamine dihydrochloride (OPD) was used as the chromogen. The reaction was analyzed at 490 nm with an ELx800 microplate reader (BioTek, Winooski, VT, USA). The results of each group of plates was standardized using a panel of reference IgG negatives and positives. P/N ratios > 2 were considered as positive antibody.

2.8 Assessment of anti-SVV antibodies in mink sera by ELISA

Heilongjiang province is located in the northeast of China, cold temperate zone and temperate continental monsoon climate, which was suitable for the mink to survive and breeding. Between June 2021 and May 2022, a total of 31 mixed samples from 4 mink farms located in the Heilongjiang province were collected. One of farm was foundation seed of mink farm, the minks were all introduced from Denmark in 2019 as SVV-negative serum samples. The blood was collected through jugular puncture from each mink with vacutainer tubes. In the field, the blood was allowed to clot before transportation to the laboratory in the district. At the district laboratory, the samples were centrifugated for 10 min at 3000 rpm to obtain the sera. Briefly, the VP2 gene were cloned into p-Cold plasmid bearing SVV structural protein expression, which was used to build the ELISA method for detecting SVV infecting antibodies. The negative-control sera showed no detectable VP2-specific antibodies in the ELISA.

2.9 Statistics

All the data between the different groups were determined with one-way repeated-measures analysis of variance (ANOVA) and the least significance difference (LSD) test. Differences were considered statistically significant at $p < 0.05$.

3.Results

3.1 Isolation and punification of a strain of SVV

The SVV isolation, Teschovirus A, Sapelovirus A (SVA), Enterovirus G, FMD, VS and SVD virus were not be detected in the vesicular outbreak clinical samples with the RT-RCR method, it was showed the expected 542-bp product size in all vesicular fluid, swab from the pig. With two sequential passages in BHK-21 cells, the SVV were successfully isolated from vesicular fluid samples. The two days post-inoculation, CPEs of infectious BHK-21 cell were found as cell lyses. No CPEs were evident in the

cell negative control (Figure1A). Merely, Senecavirus could be detected in cell with RT-PCR, and the other viruses were not detected in BHK-21. One-step growth curve of SVV-CH-09-2018 on BHK-21 cells was further examined. The multiplicity of infection (MOI) of 0.1 and 0.5 were used to infect the BHK-21 cell, and the infected cells were collected at 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 hpi, which were examined the titers with the 50% tissue culture infective dose (TCID50) assay [10] .The virus replicateed quickly at the 4 hpi, and peaked the highest titers at the 32 hpi. The maximum viral titer was TCID50/mL(Figure.1B)[11]. After infecting 24 hours, the morphology of virions in the BHK-21 were round and had a diameter of approximately 30 nm with electron microscopy(Figure. 1A).

3.2 The sequence of SVV-CH-09-2018 and phylogenetic analysis

In our study the evolutionary tree is drawn and analyzed by MEGA7.0 and OMICSTUDIO evolutionary tree software. We built this genome schema map based on prototype SVV-CH-09-2018 isolated by our lab in the Figure2. The structure of the genome includes leader protein, the 5' and 3' UTRs, the P1 region proteins, the P2 region proteins and the P3 region proteins[17]. The P1 protein constitutes the Capsid proteins and the P2p3 proteins constitute the nonstructural proteins [18]. The evolutionary tree was shown that SVV-CH-09-2018 has the typical L-4 genome layout of picornavirus. The previous study of phylogenetic analysis was shown that SVV strains could also be divided into four branches. The SVV-CH-09-2018 strain belongs to clade III.[19] The SVV-CH-09-2018 shares the highest homology of the two countries, such as American Senecavirus A strain HB-CH-2016 (Genbank, KX377924.1) (99.66%) and China Senecavirus A strain CH-01-2015 (99.67%) (Genbank, KT321458 .1).

The tree mainly screened for the Seneca genes from the United States, China, Canada, Vietnam, Colombia, Brazil, which mean that the Seneca virus was not endemic disease in a small region, but in worldwide[20].In order to indicate the relationship of various strains in the different countries clearly, we used different color to mark different strains from different countries(Figure 2A). From the tree, it was shown the spread of global SVV genomes in three major evolutionary clusters, USA- China- and Canada-like strain clusters[19]. To date, more than half of the SVV in China have been affected by SVV in US, and a part of SVV in China was from the US and Canada strain. In order

to get a clearer picture of how the Seneca virus spread around the world, we have compiled a Chronology of Landmark Incidents about SVV. The chronology was combined with the evolutionary tree in order to facilitate the analysis of the evolution and prevalence of the Seneca molecular epidemiology study of SVV[21]. As shown in Figure 2 and Table 2 , the strains isolated by our lab were separated in different location and dates, which mean that the geographic distribution and infectious host may contribute to the codon usage pattern in the evolution of SVA[22] . The geographic distribution and the host have been the main two mutational pressure with natural selection[23]. However, the location where the strain in this study came from was not clear. Based on all of these events, the pathogenicity has been stronger in a turning point for the epidemiology of the Seneca virus infection in 2015([24]), with many important features of Seneca viruses for the morbidity and mortality rates associated with the infection. The strain of this study has been for some time and continuously evolved in China. Further investigations including identification of the pathogenesis, molecular epidemiology were urgently needed to study in the evolution and prevalence of the SVV.

3.3 The mink infection of SVV

The challenge experiments were performed in mink for 5 days. There was no dead mink after viral infection through the experimental process. The pathological results did not indicate frequent gross changes, such as the surface of the lungs, ulcerative lesions, liver and kidney lesions. But the liquid faeces was found in the second days after infected SVV, which indicated the diarrhea in the small intestine of mink. All the tissues were examined with RT-PCR and RT-qPCR to test the nucleic acid of SVV[25]. The RT-PCR results were shown that oral fluid of mink (2/3) were positive, and all the fecal swab sample were positive[15]. The RT-qPCR method has been used for the detection RNA of SVV. The RT-qPCR results were corresponding to the RT-PCR in the nucleic acid level of SVV. However, the sample of fecal swabs were found much higher number of RNA copies than the oral fluid sample in the infectious groups. There was no positive sample in the control group with the RT-PCR and RT-qPCR (Table 3).

3.4 Pathological and Immunofluorescence of SVV infection

Microscopic examination revealed obvious differences among the five groups of minks with respect to their small intestine samples taken from different intestinal segments, such as duodenum and colon. The principal histopathological results are graphically summarized in Figure3A[26]. The intestinal section indicated that lesions were observed atrophy and rupture both in duodenum and colon. Followed by fusion of villi in the duodenum and colon, which were also found inflammation in different intestinal segments[27]. And the duodenum of infectious group was much more severity comparing with the control group. In addition, there were necrosis and vacuolization of epithelial cell in minks with clinical manifestations of diarrhea. The section both of duodenum and colon in control group did not have any pathologic change[28] .

The distribution and quantity of SVV virion in small intestine of mink was shown in the Figure3B. The antigens of SVV virion were identified by the multi-antibody of VP1 produced by our lab. The viral antigens were mainly detected with villous epithelial cells both in the duodenum and colon. We did not find the viral antigens in the duodenum and colon of control group. However, the duodenum and colon in the infectious groups were detected the viral antigens, and different intestinal segments contained with different virus titers. The colon was much higher titers than the duodenum with the SVV infection, which was shown that the epithelial cells of colon was much more sensitivity comparing with the duodenum with the SVV infection.

3.5 The quality of SVV specific antibody

All minks were fed in the same conditions. Their clinical symptoms were monitored daily for 28 days. On 0, 7, 14, 21, or 28 days after the challenge (d.p.c.), mink serum was collected to measure the anti-SVV neutralizing antibody titer in the two groups. (Figure3C). The SVV antibodies in the infected group were detected and measured in 28 days. There was no difference in the negative group with the antibodies. In the infectious group, the antibodies were gradually increase from the 7 to 21 days. The titer of antibody reached the peak in 21 days. And the titer had no significant decrease in the infectious group.

3.6 Serological results

The sensitivity and specificity of the VP2 from SVV were used to compare ELISA test by using the 32 clinical serum samples from 4 mink farm, one of which was negative mink farm (Table 4). Overall, 48.2% (14 out of 29) of the tested sera were reactive to the SVV recombinant VP2 antigen. The proportions of three mink farm were 0 %, 60 %, and 100% respectively. One could attribute the occurrence of the SVV reactive humoral response to the similarity VP2 - proteins. There were significant differences in the levels of antibodies detected by ELISA in the serum samples collected at different farmed mink. This observation not only supports the existence of a preexisting cross immunity in the mink farm. However, a study showed the SVV could transboundary spread from the pig to the mink. In our study, the sera used were from healthy subjects with no signs of infection, 14 out of the 29 SVV reactive sera were positives for anti - SVV IgG. The main reason was that the farmed mink is also highly susceptible to infection by different virus, often the proportion of infected mink that show clinical disease is low.

4.Discussion

Senecavirus A (SVA), was one of the vesicular diseases in pigs known as porcine idiopathic vesicular disease, which took essential role with clinic and economy in the farm animals. Recently, SVV outbreaks have been reported in numerous huge swine-producing countries. It was similar with other important vesicular virus, including as vesicular stomatitis, swine vesicular disease and foot-and-mouth disease[4]. The SVV have received much special attention, focusing on the understanding of the pathogenesis, immunology, and epidemiology of SVV, which have resulted many characters in pathogenesis of epithelial and epidermis cell, the immunosuppression, immune evasion and cross-host transmission.

In 2015, the first outbreak of vesicular lesions from newborn piglets were observed at farms in Guangdong Province of China[29], presenting with high mortality, and was diagnosed with SVA infection. To date, more than half of the province have been affected by the SVV infection in China, and our isolation strain which came from the most northern province of China was the first research. The SVV have been identified for thirty years since the first report from the US, and the turning point of the several outbreaks with SVV-vesicular disease (SVA- VD) and epidemic transient neonatal

losses (ETNL) happened in 2015[4, 30]. The first detected isolation have not been pathogenicity and clinical sign before 2010, while isolated strains are considered “contemporary” with the vesicular lesion after 2015. Recently, in the phylogenetic study, isolation SVA in a great divergence of 5.59%, the strains of our study compared with the isolation before 2010, therefore, SVV strains isolated before 2010 are considered “historical”.

The Seneca Valley virus (SVV-001) was firstly detected in a PER.C6 fetal retinoblast cell culture in 2002, which is believed that the virus was regarded as a contaminant from the bovine serum or porcine trypsin in the cell culture [31]. The SVV have experienced a great change in the nucleotide over the past ten years, and the SVV have also been found in different host and tumor cell. Mutational pressure from several animal hosts accelerates the frequency of recombinant mutations in SVV[32], cross-host transmission may have led to a rapid increase in the rate at which mutant stress is having an effect.(Chen et al., 2017)

In the pathogenesis, different types of SVV, even though which have the similar sequences in USA strains, whereas the SVV still have different pathogenicity to pigs[29]. However, the replicating efficiency in different strains were all similar high titer. These characteristics imply that the SVV have the potency to infect various host animals. Notably, SVV was detected and isolated from pigs, environmental samples, mouse feces, and mouse small intestine, even though the RNA of SVV was also detected in houseflies from negative farms of SVV far from the farm with vesicular disease[33].In 2012, there was a report shown that the SVV was with the presence of vesicular lesions and a spontaneous outbreak after being purchased at the Indiana State Fair. In 2015, the China was first detected the SVV, and which was outbreak in the 2016. The SVV strain in China have been the third major evolutionary clusters comparing with the US and Canada in the worldwide[30]. Additionally, all strains isolated from China could group into the clusters of US and Canada. As shown in Figure 2A, the isolation strain was mainly belongs to the US-like cluster.

The mink was firstly used to infect the SVV in our study, the oral fluid and fecal swab were detected the RNA of SVV with the RT-PCR and RT-qPCR methods. The mink has been the forth infectious host in the world comparing with the human, swine and mice[34]. The RT-qPCR results were indicated that fecal swab had much more quality of mRNA with SVV than the oral fluid. The pathogenesis and clinic signs were also

shown that the intestinal tract had the pathologic change, and there was no vesicular lesions after the SVV infection in the minks. Histologically, the finisher pigs had multifocal pathologic change, such as infiltration of inflammatory cells, necrotic keratinocytes and hemorrhage. Clinical evaluation in the finisher pigs has also shown that the virus can be present subclinically signs, and some did not have the clinically signs. The infiltration of inflammatory cells and the necrotic keratinocytes have been found in the experimentally infected pigs. In addition, the histopathologic lesions in the piglets were more serious than the finisher pigs, included interstitial pneumonia and ballooning degeneration of the urinary bladder and renal pelvis epithelium[5]. All of these histopathologic change reminded that the SVV would invade the epithelium and epidermis cell in the mammal, such as the pig and the mink. However, we still did not understand the clear mechanism how the SVV infect the intestinal epithelial cell and oral epithelial cells in mink.

The risk of SVA infection varies greatly between herd and farm, which include many riskers, such as high number of breeding females, more employees, and the time of weaning may contribute to the spread of SVV[35]. The analysis of serology in animal indicated that 27 out of 71 porcine samples were detected the neutralizing antibodies to SVV, 10 out of 30 bovine samples, 5 out of 35 wild mouse samples, the amount of neutralizing antibodies detected was no more than 100 human serum samples[36]. Taken together, these data were shown that SVV could naturally replicate in farm animals and human beings, and farm animals could be stimulated to produce the neutralizing antibodies[37]. But human was relatively rare in producing neutralizing antibodies. The virus shedding could be detected up to 28 days post-infection. However, studies have demonstrated that persistent infection of virus shedding could be sustained up to 60 days post SVV infection[38]. The finisher pigs produced the neutralizing antibodies at 5 dpi in experimentally inoculation, and with maximum antibody concentration between 7 and 14 dpi. But the increment of the neutralizing antibodies was decreased during the first two weeks post-infection[21]. In a longitudinal study on SVA-infected farms, the antibody titers of piglets were higher during the first week of age, but which disappeared in the last four and five weeks. More importantly, a varying from 20% to 40% in piglets with the neutralizing antibodies presented viremia and viral shedding in feces and oral fluids, which have sustained between four and five weeks without the clinical status[35]. In addition, the research found that the samples collected

from swine and their environments at several sites were shown that high genetic diversity occurs in SVA over 12 months[39]. The special immune and infection status promoted the mutation pressure, which played the main driver of the evolution of SVV rather than natural selection.

The sample of clinical evaluation from the swabs of internal and external surfaces on the farm was found the nucleic acid of SVV, which mean that the SVV has been the environmental risker. The detection of SVA in mice and housefly indicated that these may play a role on the epidemiology of SVV, which would also increase the risk of SVV infecting the wild animal being as natural host[33], and mice may act as a natural reservoir and potential vector. In another hands, the minks were at the top of food chain in the mice, which could add the opportunity of the SVV infection and the evolution of viruses. Previously, the mutational pressure is considered as the major factor in the variation compared with natural selection. Most of the studies were focused on the geographic distribution contributing to the codon usage pattern of SVA, and the mutational pressure played a more important role in the SVA evolution compared with natural selection[40]. However, there was no research focused on the cross-species transmission, such as the complex links between physiological differences of hosts, disease progression and viral release. The mink infected the SVV provided a new factor in the mutational pressure, which would speed the understanding of the SVV in the cross-species transmission and viral life cycle within the environment, and human could block the host in the spread of SVV. All of these studies would increase the scientific proof for preventive measures against SVV.

The SVV could formulate the immune evasion not only in the immune system of human being, but in animals. The antibodies of surface antigen showed that the SVV could stimulate the immune system of mink, and antibodies titer was increased with the mink infected SVV. In the period of clinical evaluation, IgG antibody dynamic in clinically affected and non-affected animals had no significant difference. SVV was originally identified as a potent oncolytic virus against tumors in medicine, including the features that targeting and penetrating solid tumors via intravenous administration, the inability for insertional mutagenesis, and self-replicating RNA virus with selective tropism for cancer cells[41]. The strong cellular immune response was induced with the SVV infection, which promote the IFN-γ-specific T cells respond as early as 3–7 dpi[42]. And it is reasoning that T-cell responses could not completely clear SVV at 14 dpi.

However, there was no change with the evolution SVV from the same infecting farm for one year research, which indicated that the evolution was not from one host of pig. Actually, the multiple hosts were as the mutational pressure plotted against the SVV evolution and cross-species transmission.

Conflict of interest statement

The authors declare no financial or commercial conflicts of interest.

Data availability statement

The data support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Xinpeng Jiang: Methodology, Funding acquisition. Di Liu: Methodology. Ziliang Qin: Writing – original draft. Chao Chen: Data curation. Shaojun Chen: Data curation. Gang Li: Data curation. Haijuan He: Data curation. Zida Nai: Investigation. Xinmiao He: Investigation. Wentao Wang: Investigation. Ming Tian: Investigation. Heshu Chen: Investigation. Fang Wang: Investigation.

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Figure Legend

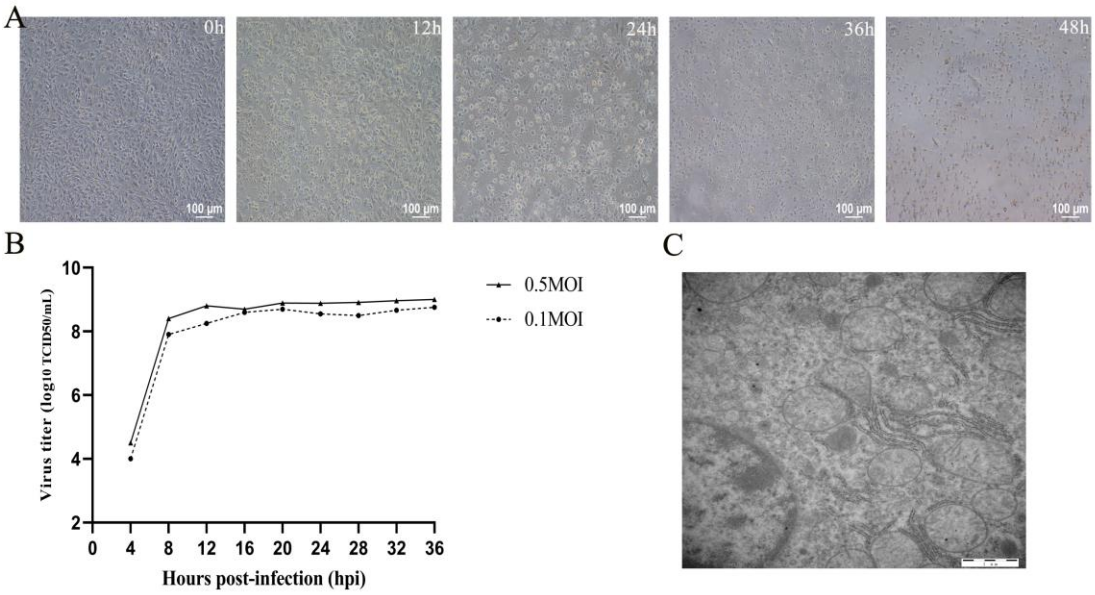

Figure 1. The process of BHK-21 cell CPEs induced by SVV-CH-09-2018 infecting cells (A) One-step growth curve of SVV-CH-09-2018 on BHK-21 cells with the multiplicity of infection (MOI) of 0.1 and 0.5 (B). An electron microscope image of the SVV virus(C)

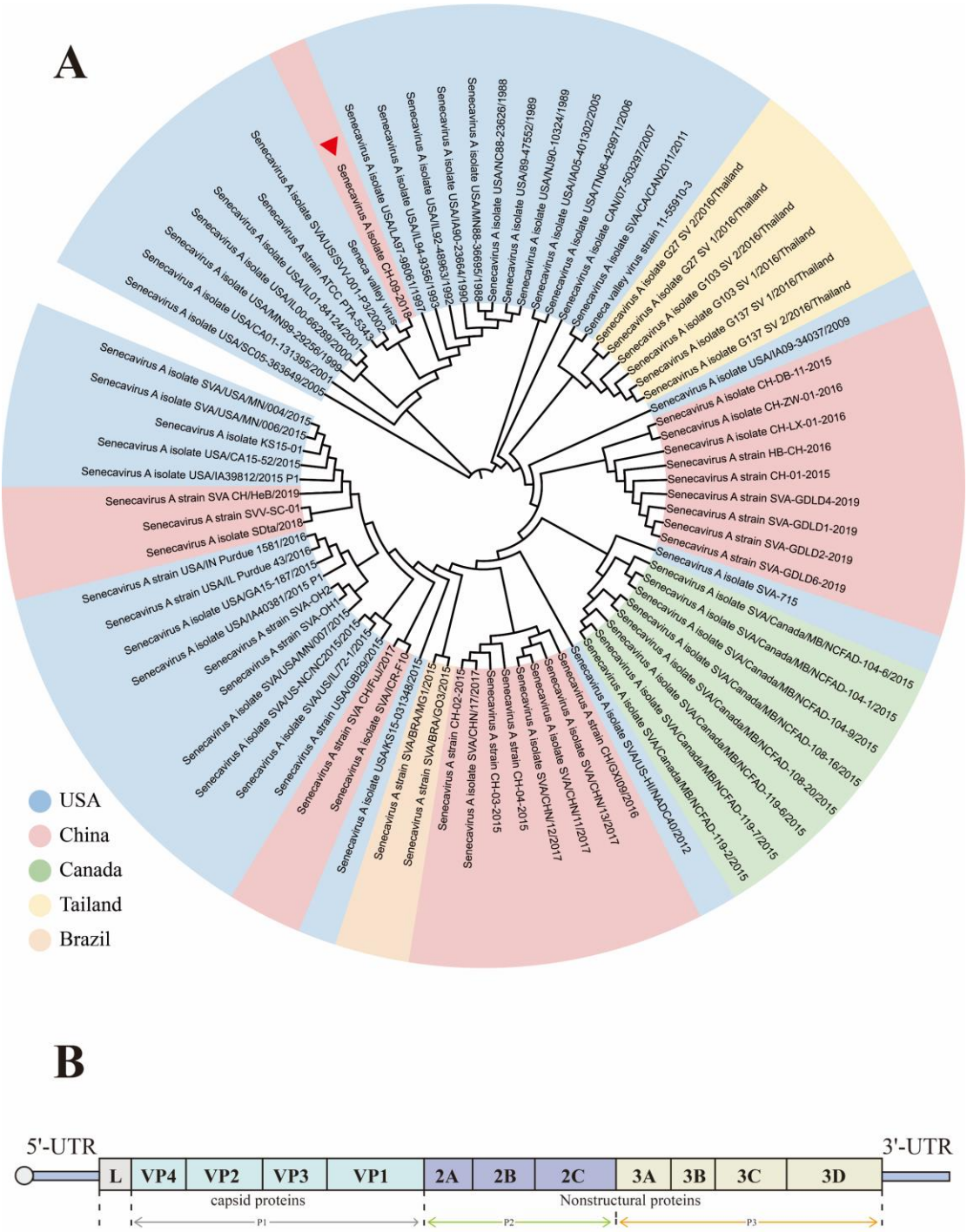

Figure 2. The evolutionary trees are drawn by Mega 7.0 and Omicstudio, the analysis data were obtained from NCBI database with the whole genome of reference SVV strains. In the final evolutionary tree, the experimental virus strain SVV-CH has been marked (▲), and the strains from different countries are also marked with different

colors (A). Genome structure of type the Seneca virus, showing the plane structure of virions. It is composed of a 5 '-terminal non-coding region (5' UTR), a 3 '-terminal non-coding region (3' UTR) encoding a polyprotein, and only one open reading frame (ORF) (B).

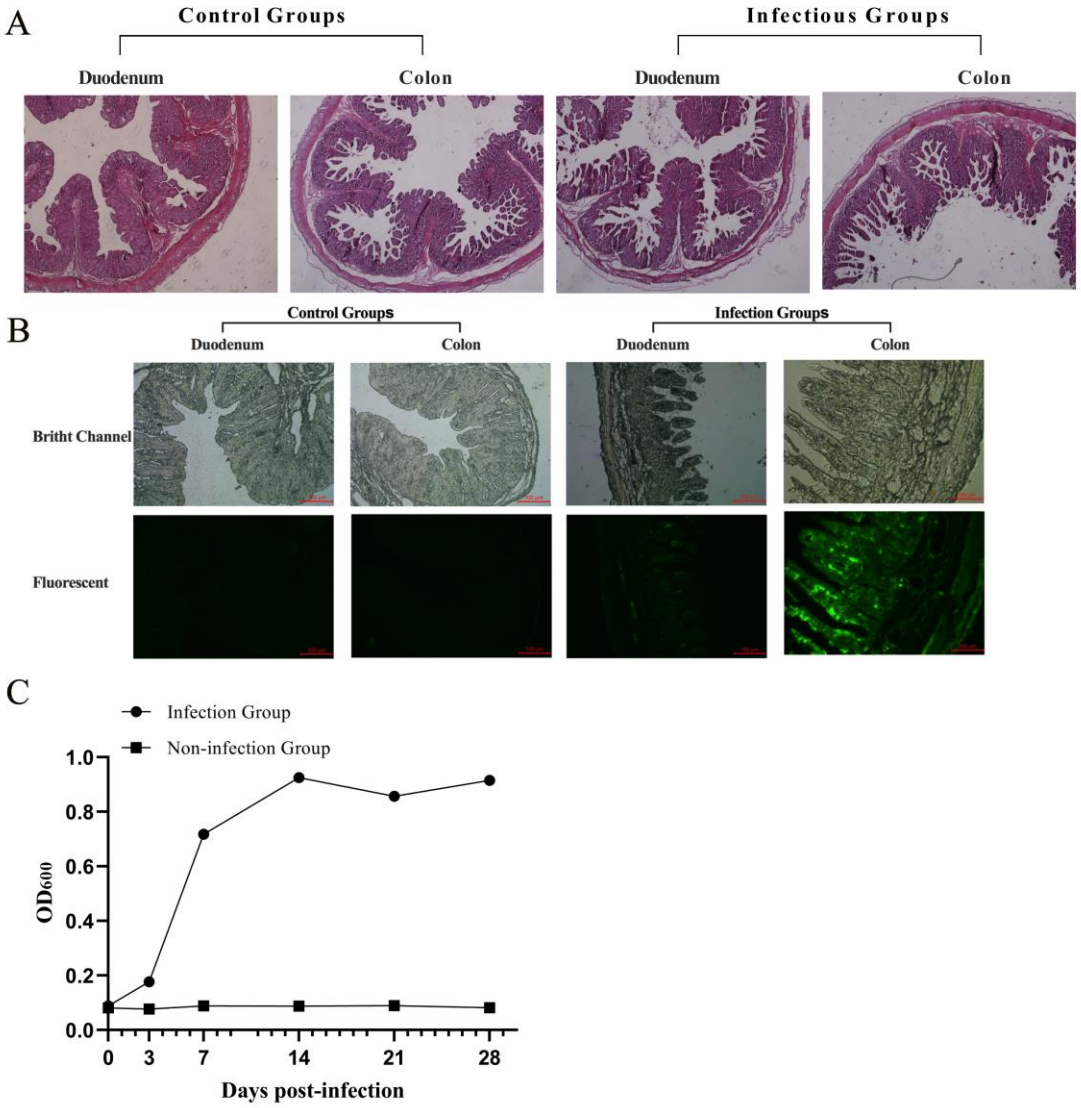

Figure 3. The pathology and immunofluorescence indicated the pathological changes and the quality of virus virion. The pathological changes of duodenum and colon after the SVV infection (A). Viral antigens were detected mainly in villous epithelial cells of the colon. and the titer of the colon was much higher than that of the duodenum (B). In the mink challenge experiment, at 0, 7, 14, 21 and 28 days after challenge, mink sera were collected to determine the level of anti-SVA neutralizing antibody (C).

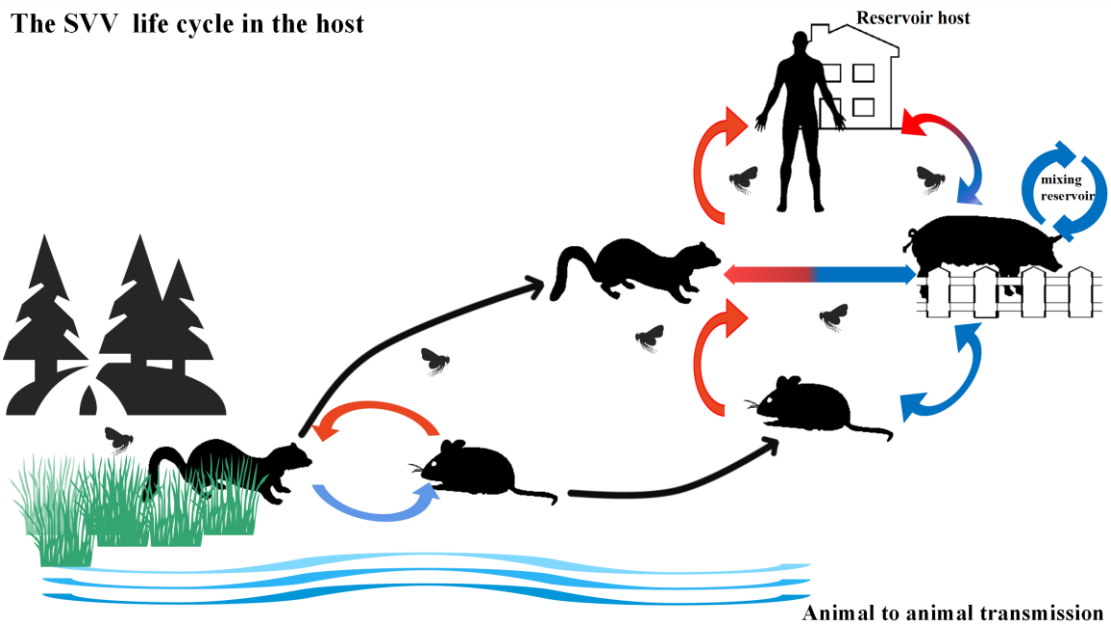

Figure 4. To indicate the adaptation and transmission of SVV. An adaptation from pig SVV to human SVV includes two circulations, the food chain circulation and the reservoir circulation. In the reservoir and food chain circulation, SVV are transmitting, mutating, and adapting between mice and minks (as well as other semiaquatic mammals). Mink and mice can be infected through contacting with epidemic water. In a free stall barn system, usually in some areas of developing countries, pig will inevitably lead to a land habitat circulation including human beings. The blue pathway is transmitted by fecal route, while the red one is transmitted by oral route. In rural areas of South Asia, Southeast Asia, Southern and Eastern China, pigs mink and mice, in particular chick are often observed to eat by human. The pigs and mink also have the opportunity to eat mice feces, but mice seldom eat pig feces.

Table 1 Primers for the amplification and identification of whole genome

primers	Sequences (5′–3′)	Positions	Size(bp)
F (1-623)	TTTGAAATGGGGGGCTGGG	1-623	623
R(1-623)	CTATCAGGCAGTATCCAAAGCACGC	1-623	
(498-1352)M13+	CGACCCAGGACTTCTTTTTGAAT	498-1352	855
(498-1352)M13-	GAGAAGGTTTTTACAGCT	498-1352	
(1336-1662)M13+	GCTGTAAAAACCTTCTC	1336-1662	327

(1336-1662)M13-	ATAGTATGTGCCAAGAG	1336-1662	
(1612-2748)M13+	GATTACCGGACCGGGAAAAACAT	1612-2748	1137
(1612-2748)M13-	ACCAGAGAAATCGGTGTCAGT	1612-2748	
(2996-4041)M13+	CTTCACTGGACTTCAATTTTTATA	2996-4041	1046
(2996-4041)M13-	CTCCAACTGGTACTGGAGGACAG	2996-4041	
(4004-4995)M13+	AAGAGAAAGCCAGCCCTGTCCTCC	4004-4995	991
(4004-4995)M13-	ACCTAGCTTGGCAAGAATAGCCAAACG	4004-4995	
(4929-5949)M13+	GGCGCTTGTCGACCTCACTCCAGA	4929-5949	1021
(4929-5949)M13-	ATCAAATTTTGACAACACAGCA	4929-5949	
(5879-6908)M13+	AATTGAGAAAGACGACCGCACA	5879-6908	1030
(5879-6908)M13-	GTCATCTTATACCCCAACTT	5879-6908	
(6881-7297)M13+	GCGCTGCCAAGTTGGGGTATAA	6881-7297	417
(6881-7297)M13+	CCTTTTCTGTTCCGACTGAGTT	6881-7297	
SVV-F	AACCGGCTGTGTTTGCTAGAG	59–79	
SVV-R	GAACTCGCAGACCACACCAA	205–186	147
SVV-P	6/FAM-TCGAGAAGCTGCAATCTG/MGB-NFQ	143–167	

Table 2 Chronology of SVV Landmark Incident

Detection	Species	Reference	Country	Incident
2002	Human (PER.C6)	[24]	USA	This virus was first discovered as a serendipitous finding in 2002, while cultivating adenovirus-5-based vectors in the cell linePER.C6
2007	Swine	[43]	Canada	In 2007, about 80% of 187 pigs shipped from Canada to the United States developed blister disease, and Senecavirus RNA was detected in these biological samples
2007	Human (Neoplasms with neuroendocrine properties)	[36]	USA	SVV-001 has potent cytolytic activity and high selectivity for tumor cell lines on neuroendocrine properties versus adult normal cells. Systemically administered SVV-001 has potential for the treatment of metastatic neuroendocrine cancers.
2008	Seneca virus (SVV-001)	[18]	USA	Complete genome sequence analysis of Seneca Valley virus-001, a novel oncolytic picornavirus
2012	Swine		USA	The United States reported the Seneca outbreak in pigs symptomatic vesicular
2015	Seneca virus	[44]		In 2015, the International Committee on the Classification of Viruses (ICTV) renamed SVV "Senecavirus A" (SVA) after the genus it belongs to, the Senecavirus.
2015	Swine			The year 2015 was a turning point in the epidemiology of infections, with the massive global outbreak of Seneca
2015	Human (Solid Tumors)	[45]	USA	The SVA as an anticancer treatment, NTX-010, in Phase I trials in children with relapsed/refractory solid tumors by Neotropix.
2015	Swine	[17]	Brazil	The Senecavirus infect outside of North America

2016	Swine	[46]	China	The First Identifification and Complete Genome of Senecavirus A affecting Pig with Idiopathic Vesicular Disease in China
2016	Swine	[47]	Colombia	Emergence and whole-genome sequence of Senecavirus A in Colombia
2016	Swine	[48, 49]	Thailand	The first detection of Senecavirus A (SVA) in pigs in Thailand.
2016	Mice and houseflies	[33]	USA	Detection of the Emerging Picornavirus Senecavirus A in Mice and houseflflies, which may play a role in SVA epidemiology
2017	Swine feed	[50]	Brazil	Seneca Valley virus RNA detection in pig feed and feed ingredients.
2018	Swine	[51]	Vietnam	First Detection and Genome Sequence of Senecavirus A in Vietnam
2018	Human	[52]	Japan	Structural basis for anthrax toxin receptor 1 recognition by Seneca Valley Virus
2018	Swine	[53]	Brazil	A new wave of Seneca Valley virus outbreaks in Brazil
2019	Swine	[54]	USA	Developed a recombinant SVA strain (rSVAm SacII) using reverse genetics and assessed its immunogenicity and protective effificacy in pigs.
Our study	Mink		China	The mink infected the SVV isolating from the pig

Table 3 The result of RT-PCR and CT ranges of qRT-PCR with positive samples

Groups	Sample identification	SVV RT-PCR			SVV qRT-PCR (C_T range)		
		Oral fluid	Serum	Fecal swab	Oral fluid (21.8–35.9)	Serum (15.3–35.7)	Fecal swab (22.5–35.2)
Infectious Group	Sample 1	(+)	—	+	34.9	35.9	25.6
	Sample 2	—	—	+	36.7	36.9	28.9
	Sample 3	(+)	—	+	35.6	36.1	23.9
Control Group	Sample 4	—	—	—	37.1	37.7	36.2
	Sample 5	—	—	—	36.2	36.6	35.9
	Sample 6	—	—	—	37.7	38.1	36.1

Table 4 Anti-SVV antibody levels in mink sera

Region	Negative	Positive	p-value
Negative farm	3（100%）	0（0）	p<0.01
Farm 1	11（100%）	0（0）	NA
Farm 2	4（40%）	6（60%）	
Farm 3	0（0）	8（100%）	