Immunogenicity and protective efficacy of an inactivated cell culture-derived Seneca Valley virus vaccine in pigs

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A B S T R A C T
Seneca Valley virus (SVV) infection in pigs is associated with porcine idiopathic vesicular disease (PIVD). Outbreaks of SVV infection in pig herds have been reported in several Asia and Americas countries. Recently, a series of outbreaks of SVV infection occurred in China, Canada, Thailand and the United States. However, no available vaccines have been developed to limit the transmission of SVV. The SVV CH-FJ-2017 from Fujian province in China is a representative of the epidemic strains, and shows 98.5–99.9% capsid protein amino acid identity with the recent SVV strains. In the present study, we developed a SVV CH-FJ-2017 inactivated vaccine. The SVV was produced by cultivation of BHK-21 cells in roller bottles, inactivated with binary ethylenimine, and mixed with oil adjuvant (Montanide ISA). The immunogenicity of the inactivated vaccine in pigs was evaluated by neutralizing test, and the immunized pigs were challenged with SVV CH-FJ-2017. The results showed that animals receiving one dose of the inactivated vaccine (2 μg/dose) with oil adjuvant developed high neutralizing antibody titers and showed no clinical signs after virus challenge comparing with the non-vaccinated animals, indicating a good protective efficacy of the produced vaccine against SVV infection. This is the first reported SVV vaccine that can be used for control of SVV infection in pigs.

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1. Introduction

Seneca Valley virus (SVV) belongs to the genus of Senecavirus within the family of Picornaviridae. SVV is a non-enveloped, non-segmented, positive-sense RNA virus with a genome size of approximate 7.2 kb [1–3]. The viral genome of SVV includes a single open reading frame (ORF) that encodes a polyprotein. The single ORF shows the typical gene arrangement of picornaviruses with the order of 5'-L-VP4-VP2-VP3-VP1-2A-2B-2C-3A-3B-3C-3D-3' [3]. The 3Cpro has been determined as the protease of SVV that can cleave various host proteins [4]. VP2 protein has been used to establish an indirect enzyme-linked immunosorbent assay (ELISA) to diagnose SVV infection [5]. The functions of other viral proteins of SVV have not been reported and the viral pathogenesis mechanisms remain unclear. Further studies should be performed to investigate the puzzles of SVV and limit SVV infection.

The infection of SVV often results in vesicular lesions at the coronary bands, hooves, or snouts in pigs [6]. As the only species in the genus Senecavirus, SVV was first isolated as a contaminant from the PER.C6 cell line in 2002 in the United States, which was deemed to be introduced into the cell culture by using contaminated fetal bovine serum or porcine trypsin [2]. SVV was initially not associated with any specific pathology and was used as an oncolytic virus to treat human cancers [7]. In 2007, SVV infection was determined to be associated with porcine Idiopathic Vesicular Disease (PIVD) occurred in Canada [8]. In 2014 and 2015, a high number of SVV infection in pigs occurred in the United States, Brazil and China [6,5–11]. In 2016 and 2017, a new round of SVV infection in pigs was subsequently reported in China, Thailand and Colombia [12–14]. This apparently suggests a quick spread of SVV infection in more countries. Meanwhile, evidences have indicated that the recent SVV strains have evolved and showed more virulent phenotype comparing with previous strains [15–17].

The persistent occurrences of SVV infection in different regions imply a potential risk of pandemic outbreak. Therefore, a series of methods and policies are required to control and limit the spread of this virus. The clinical manifestations of SVV infection resemble those of vesicular transboundary animal diseases such as foot and mouth disease (FMD), swine vesicular disease (SVD), vesicular stomatitis (VS), and vesicular exanthema of swine (VES). It is hard to directly distinguish these vesicular diseases by observation of
clinical signs. Several laboratory diagnostic methods have been developed recent years to detect SVV infection, including a indirect ELISA, a competitive ELISA (cELISA), a virus serum antibody neutralizing test and two specific real-time RT-PCR (RT-qPCR) assays [5,18–21]. These developed laboratory methods can sensitively detect SVV RNA in vesicular diagnostic tissues. However, there remain no developed vaccines that can protect pigs against SVV infection. The principal aim of this study was to develop a vaccine candidate that can elicit neutralizing antibodies against SVV and also protect pigs against SVV infection.

2. Materials and methods

2.1. Ethics statement

All the animal experiments in this study were approved and carried out according to the requirements and management guidelines of the Gansu Animal Experiments Inspectorate and the Gansu Ethical Review Committee (License No. SYXK [GAN] 2014-003).

2.2. SVV production in BHK-21 cells and pathogenesis studies of CH-FJ-2017 in pigs

A SVV strain CH-FJ-2017 (GenBank: KY74510) was isolated from Fujian, China in January 2017 by our lab previously [13]. Baby hamster kidney cells (BHK-21) were maintained in the Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and cultured at 37 °C under 5% CO₂. The propagation of SVV was performed in the roller bottles (1500 cm²) using the DMEM medium (10% FBS). The pathogenesis investigation of CH-FJ-2017 in pigs was performed as described previously [15]. Briefly, two groups of finishing pigs (with 5 pigs as infection group, and 3 pigs as control group) were challenged with 3 mL of DMEM or CH-FJ-2017 (10⁵ TCID₅₀/mL) by intranasal (1.5 mL to each nostril) routes. All the animals were monitored daily for clinical signs, and the rectal temperatures were measured daily for 12 days. The clinical scores were used to evaluate the clinical signs of the disease. Clinical signs were scored as follows: the asymptomatic animals, 0 point; each foot bearing lesions, 1 point; vesicular lesions in or around the mouth, 1 point. Therefore, the maximum score per animal was 5. The rectal temperature higher than 40 °C was deemed to have developed a fever.

2.3. Virus inactivation

For SVV inactivation, the supernatants from the SVV-infected BHK-21 cultures in the cell culture flask were collected and clarified. The clarified supernatants were inoculated to the roller bottle cultures and maintained until 100% cytopathic effect was observed. The viral cultures were then collected and clarified. The aziridine compound binary ethylenimine (BEI) was used to inactivate the prepared viral antigens. The complete inactivation of the antigen was confirmed by incubation of the antigen in the BHK-21 cells and then passaging for 3 times, and the viral RNA and cytopathic effect (CPE) was determined.

2.4. Formulation of inactivated vaccine with oil adjuvant

For the formulation of inactivated vaccine, the inactivated antigens were purified by a sucrose density gradient and the oil adjuvant (Montanide ISA 206 [Seppic]) was used as the adjuvant. The sucrose density gradient centrifugation was performed as previously described [22]. The antigens were purified from others by separating them on linear 15–45% sucrose density gradients after centrifugation at 35 000 rpm at 4 °C for 180 min. Each fraction from such gradient was measured at a wavelength of 260 nm. The majority of the antigens were found in the 30% layer of the sucrose density gradient. The virions were also observed by electron microscopy as described previously [23]. The antigens were desugared and diluted, and the concentration was measured by the BCA protein assay kit. The water-in-oil (W/O) CH-FJ-2017 vaccine was produced containing BEI-inactivated SVV antigen as previously described [24]. Equal volumes of oil adjuvant Montanide ISA 206 was added to equal amounts of aqueous phase (50:50). The aqueous phase contained 100 μg of antigens. The mixture was stirred to form the W/O vaccine.

2.5. Animals and immunization experiments

A total of 21 finishing pigs were randomly divided into 4 groups, with each 6 pigs in group 1, 2 and 3, and with 3 pigs in group 4. The pigs in group 1, 2 and 3 were immunized intramuscularly in the neck with one dose (2 mL, 2 μg), 1/3 dose (0.67 μg antigen) and 1/9 dose (0.22 μg antigen) of vaccines respectively. The pigs in group 4 were non-vaccinated and used as the negative controls. The clinical signs of the pigs and the RNAemia were investigated to evaluate the safety of the vaccine. The serum samples were collected at the 1, 3, 5, 7, 9, 14, 21, 28, 35 and 42 days post-vaccination (dpv). The vaccine potency was evaluated by neutralization test.

2.6. Immunogenicity assessment (Neutralization test)

The neutralizing antibody titers in the serum of the pig immunized with the vaccines were detected by virus neutralizing antibody test (VNT). Antibody titers required for neutralization of SVV CH-FJ-2017 were determined using BHK-21 cells as described previously [25,26]. Briefly, the pig serum was diluted in a two-fold serial dilution and added into the 96-well tissue culture plate (50 μl each well). The 50 μl of 200 TCID₅₀ SVV CH-FJ-2017 was added to each well. After incubation at 37 °C for 1 h, 50 μl of 10⁵ cells/mL BHK-21 cell in MEM containing 8% FBS were added to each well. Endpoint titers were determined at 72 h post-infection (hpi) and expressed as the reciprocal of the final serum dilution that resulted in the neutralization of the virus activity by 50%.

2.7. Protection against virus challenge

At the 42 dpv, all the vaccinated and non-vaccinated pigs were inoculated with 3 mL of CH-FJ-2017 (10⁵ TCID₅₀/mL) by intranasal (1.5 mL to each nostril) routes [15]. To investigate the protective efficacy of the vaccine, all the challenged pigs were monitored daily for clinical signs, and the rectal temperatures were measured daily for 12 days. Blood samples were collected daily after the virus challenge. The viral RNA copy in the blood was detected by quantitative real-time PCR method as described previously [19]. The RNA copy numbers were determined by an established standard curve, and the results were presented as log₁₀RNA copies/100 μl.

3. Results

3.1. Similarity of P1 amino acids sequences of the SVV CH-FJ-2017 strain and other reported SVV strains

SVV CH-FJ-2017 is a representative of the recent epidemic strains that was isolated in China in 2017 and showed close phylogenetic relationship with current SVV strains [13]. To analyze whether CH-FJ-2017 potentially matches the other circulating SVV strains and could be used as a vaccine candidate, an analysis of the P1 (VP4-VP2-VP3-VP1) amino acid sequence of CH-FJ-2017
with other SVV strains in different countries available in GenBank was performed. As shown in Table 1, the results indicated that SVV CH-FJ-2017 showed 98.48–99.88% P1 similarity with the recent SVV strains, and showed 97.20% similarity with the prototype SVV strain (SVV-001) [3,12]. Therefore, the results indicate that CH-FJ-2017 shows extremely high amino acids similarity with the recent circulating SVV strains. Although the vaccine matching assay should be further investigated, we speculated that it might be a potential vaccine candidate to control of SVV infection.

Table 1
The P1 amino acids similarity of CH-FJ-2017 with other SVV strains.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Host</th>
<th>P1 amino acid similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA strains</td>
<td>Pig</td>
<td>98.72–99.65%</td>
</tr>
<tr>
<td>Canada strains</td>
<td>Pig</td>
<td>98.83–99.53%</td>
</tr>
<tr>
<td>China strains</td>
<td>Pig</td>
<td>98.60–99.88%</td>
</tr>
<tr>
<td>Brazil strains</td>
<td>Pig</td>
<td>99.42–99.53%</td>
</tr>
<tr>
<td>Thailand strain</td>
<td>Pig</td>
<td>98.48%</td>
</tr>
<tr>
<td>Colombia strain</td>
<td>Pig</td>
<td>99.53%</td>
</tr>
<tr>
<td>SVV-001 strain</td>
<td>PER.C6 cell line</td>
<td>97.20%</td>
</tr>
</tbody>
</table>

3.2. One-step growth curve of CH-FJ-2017 on BHK-21 cells and the clinical manifestations in pigs

BHK-21 cells were infected by CH-FJ-2017 at a multiplicity of infection (MOI) of 0.1 or 0.5, and the infected cells were collected at 0, 4, 8, 12, 16, 20, 24, 30 and 36 hpi. The viral loads were titrated by 50% tissue culture infective dose (TCID_{50}) assay. The virus quickly replicates at 4 hpi, and reached a maximal constant value at 30 hpi. The maximum viral titer was about 10^{8.33}TCID_{50}/50ul (Fig. 1B). The clinical manifestations of CH-FJ-2017 in pigs were subsequently determined as described previously [15]. The detailed viral challenge method was described in the Methods section above. All the SVV challenged pigs showed significant clinical diseases after 2–4 days post-challenge (dpc), including fluid-filled vesicles on the snout and ulcerative lesions on the coronary band. No clinical signs were observed in the negative control pigs. The clinical signs of the pigs with PIVD were described using the clinical scores and the viral RNAemia was detected by qPCR (Fig. 1C). The viral RNA can be detected in the blood after 1–7 dpc; and the RNAemia disappeared after 9 dpc. Three of five pigs developed fever at 2–6 dpc (#PC28, #PC20 and #PC19), and the temperatures return to normal after 1–3 days of fever.

3.3. Immunogenicity of the inactivated CH-FJ-2017 vaccine in pigs

Virions of SVV were examined by electron microscopy before formulation of inactivated vaccine and the images were recorded. The virions were round and had a diameter of approximately 30 nm (Fig. 1D). This confirmed the purification of the antigens, and the vaccine was subsequently formulated. The complete inactivation of the antigen was confirmed by incubation of the antigens in BHK-21 cells and then passaging for 3 times. No CPE was observed and no viral RNA was detected after three times passage (data not shown). This confirmed the complete inactivation of the
To further evaluate the protective efficacy of the inactivated CH-FJ-2017 vaccine, the four groups of immunized or control pigs were challenged with CH-FJ-2017 (10^9 TCID50/mL) at 42 dpv. Upon challenge, the clinical signs and lesions were monitored and recorded. As shown in Fig. 2, in the 3 non-vaccinated pigs (control group), significant clinical diseases were observed in all 3 pigs after 2–3 dpc, and two of the pigs developed fever. The RNAemia could not be detected in all of 3 pigs and the RNAemia disappeared at 9 dpc. In the full dose of vaccine given group, all the vaccinated pigs showed no clinical signs and fever, No. PC1 showed low level of RNAemia; other 4 pigs developed no clinical signs and fever. In the 1/3 dose vaccinated pigs, pigs No. PC1 and No. PC3 showed mild clinical diseases, and No. PC1 also developed fever, No. PC3 showed low level of RNAemia; other 4 pigs developed no clinical signs and fever. In the 1/9 dose group, two pigs showed no clinical signs and no RNAemia (No. PC32 and PC36); one pig showed significant clinical signs of disease with fever (No. PC38); one pig showed low level of with fever (No. PC37); and two pigs developed fever with no other signs (No. PC34 and PC35).

4. Discussion

Vaccination plays a significant role in the prevention and control of various old and newly-emerged contagious diseases [27]. SVV infection in pigs shows significant potential impact on the productivity and economics of the pork industry [28]. The persistent and long-term circulation, spread and evolution of SVV will make the control of the disease more difficult and complicated. In China, SVV has been detected continuously in 2015, 2016 and 2017 [9,13]. The possibility of SVV become a potentially endemic virus in some pig farms or causing subclinical infections should be studied and monitored at present in China. Besides, the SVV has been detected and isolated from mice, mouse feces and environment samples, SVV nucleic acid was also detected in houseflies, suggesting a highly contagious characteristic of this disease [29].

FMD with similar clinical manifestation as SVV infection is being controlled by using the inactivated vaccine [30]. Inactivated vaccines do not contain any live components, and therefore, being safe for less risk of inducing the disease [31]. To provide an available vaccine that could be used to control of the SVV transmission, we developed an inactivated SVV W/O vaccine. The SVV CH-FJ-2017 was cultivated in BHK-21 cell cultures, and was inactivated by BEI. The inactivated SVV experimental vaccines showed good immunogenicity and economics of the pork industry [28].
Immunization of the inactivated vaccine did not result in a detectable RNAemia and observed clinical signs in the vaccinated pigs which confirm the safety of the vaccine. It suggests that the SVV CH-FJ-2017 strain is a potential vaccine candidate, and it could be used to limit the current SVV strains in China. The analysis of P1 amino acids region of CH-FJ-2017 suggests that this SVV strain shows high protein similarity with other circulating SVV strains (Table 1). The polyprotein P1 region includes the four structural proteins (capsid proteins) of SVV that are responsible for induction of the neutralizing antibodies in the host. SVV has only one serotype, the high amino acids identity of the P1 between CH-FJ-2017 with other SVV strains suggests that the developed vaccine candidate might match many of the circulating strains in different countries, and implies the potential protective ability of the produced vaccine against other different SVV strains. Meanwhile, our previous study found that most of the determined SVV strains were clustered in a same clade with CH-FJ-2017; this further implies the potential efficacy of CH-FJ-2017 vaccine against the circulating SVV strains. However, this is only a speculation at present; the antigenic matching assays should be further performed to investigate the efficacy.

In conclusion, our study has developed a SVV vaccine candidate that was immunogenic and safe in pigs, and proved that the developed vaccine has good protection against SVV infection. This vaccine candidate can be potentially used for limiting the transmission of SVV.

Potential conflict of interests

No reported conflicts in all authors.

Acknowledgments

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Fig. 2. Protective efficacy of the CH-FJ-2017 vaccine in pigs. Pigs were vaccinated with 1, 1/3 or 1/9 dose of vaccines prepared from CH-FJ-2017 and challenged 42 days later. The clinical signs were monitored daily and the rectal temperature and RNAemia were determined.

References


