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

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ABSTRACT

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.

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 $3C^{pro}$ 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 [6,9–11]. 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

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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'smedium (DMEM) supplemented with 10% fetal bovine serum (FBS) and cultured at 37 °C under 5% CO_2. The propagation of SVV was performed in the roller bottles (1500 cm^2) 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 (10^9TCID_{50}/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 $TCID_{50}$ SVV CH-FJ-2017 was added to each well. After incubation at 37 °C for 1 h, 50 μl of 10^6 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 (10^9TCID_{50}/mL) by intranasal (1.5 mL to each nostril) routes [15]. To investigate the protective efficiency 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 log_{10}RNA 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

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