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Efficacy of an inactivated *Senecavirus A* vaccine in weaned pigs and mature sows

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Introduction

SVA is a non-enveloped, single-stranded, positive-sense RNA virus (~7.2 kb) that is the only member of the genus *Senecavirus* in the family *Picornviridae* [1]. Other swine viruses in the family *Picornaviridae* include foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV). SVA has one large open reading frame that encodes a single long polyprotein cut by viral proteases in the standard L-4-3-4 format of picornaviruses resulting in 12 mature proteins: leader protein, VP4, VP2, VP3, VP1, 2A, 2B, 2C, 3A, 3B, 3C, 3D [1]. The structural proteins, VP1-4, form protomers that make up the icosahedral capsid of the virion. Linear B cell epitopes have been identified on both VP1 and VP2 proteins using monoclonal antibodies [2]. Early IgM and IgG responses have been demonstrated to be primarily directed against VP2 and VP3 [3].

Late 2014 and into 2015, Brazil had a spike in idiopathic vesicular disease cases and increased neonatal mortality documented in the first week of life [4]. Samples collected from these farms tested PCR positive for SVA [5]. Shortly after, the US saw similar cases of vesicular disease and neonatal mortality attributed to SVA starting the summer of 2015 [6-8]. Contrary to attempts in the past, multiple groups were able to experimentally reproduce clinical disease in pigs of various ages demonstrating that SVA is a causative agent for vesicular disease in swine [9-11]. Subsequently, other countries including Canada [12], China [13], Colombia [14], Thailand [15], and Vietnam [16] reported vesicular disease cases due to SVA.

There are multiple viruses that infect swine and cause vesicular disease including: SVA, FMDV, SVDV, vesicular stomatitis, and vesicular exanthema of swine. Vesicular disease caused by each of these viruses is grossly identical and further diagnostics must be performed to differentiate the cause of disease [5]. Differentiating the cause of vesicular disease in swine is important because foot-and mouth disease (FMD) caused by FMDV is on the World Organization for Animal Health (OIE) list of notifiable diseases [17]. For countries with FMD negative status, identifying a case of FMD would have severe economic ramifications including production loss, trade restrictions, and cost of regaining FMD-free status [18]. With more FMD-free countries identifying SVA, there is also an economic impact of an investigation to rule out FMDV every time an animal with vesicular disease is identified. A vaccine for SVA could reduce the occurrence of SVA-related vesicular disease thus improving swine welfare and reduce the economic burden of an endemic vesicular disease in FMD-free countries.

Research groups have previously shown the efficacy of a live-attenuated vaccine [19] and an inactivated vaccine [20, 21] for protection against clinical disease of SVA in weaned pigs and growing pigs. Recently there was also a publication evaluating an inactivated SVA vaccine in gestating sows and the levels of maternally derived neutralizing antibodies in milk and persistence in piglets. Expanding these previous studies, the objectives of this research were to not only evaluate efficacy of an inactivated SVA vaccine in weaned pigs and mature sows, but also the protection provided to piglets by maternal immunity from immunized dams. In addition, the virus utilized to challenge the gestating sows and piglets was specifically isolated from a SVA positive farm experiencing increased neonatal mortality.

Materials and Methods

Ethics statement

The animal work in this study was performed under approved animal care and use protocols (ARS-2867, ARS-2019-793) by the National Animal Disease Center's Institutional Animal Care and Use Committee. All animals were euthanized at the end of the study with an intravenous administration of a barbiturate (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) following the label dose (1 mL/4.54 kg).

Virus inactivation and vaccine formulation

An SVA strain isolated in 2015 from a clinical case of vesicular disease in pigs from Iowa (SVA/2015/IA) was propagated on swine testicular (ST) cells cultured at 37 °C and 5% CO_2 in minimum essential medium (MEM) supplemented with 2.5% HEPES (Gibco, Thermo Fisher Scientific, Waltham, MA). Twenty-four hours after cell cultures were inoculated, supernatant was collected and clarified by 0.2 µM filtration. Pre-inactivation, the virus titer ranged between 4-8x10^8 log $TCID_{50}$/mL. Binary ethylenimine (BEI) in addition to formalin were used to ensure inactivation of the virus. Inactivated viral solution was neutralized with sodium thiosulfate and sodium bisulfite. Inactivation was confirmed by multiple passages in cell culture.

Inactivated virus was mixed with an oil-in-water adjuvant at 12.5% v/v (87.5% v/v antigen). Antigen and adjuvant were mixed for 30 min at room temperature and stored at 4°C prior to use. Vaccine was mixed gently directly prior to being drawn up into syringes for administration.

Challenge viruses

A SVA strain was isolated from a clinical case of vesicular disease in finishing pigs from South Dakota in 2015 (SVA/SD/2015) [6]. The isolate was propagated on ST cells cultured at 37 °C and 5% CO2 in MEM (MilliporeSigma, St. Louis, MO) supplemented with 10% FBS (AtlantaBio, Flowery Way, GA), gentamicin (VetOne/MWI, Boise, ID) and L-glutamine (Life Technologies, Carlsbad, CA). The virus was passaged 4 times in cell culture and was diluted to a titer of 1x10^7 $TCID_{50}$/mL with MEM. Titrations were calculated using the Reed and Muench method [22].

A second SVA strain was isolated from piglet intestine from a farm experiencing vesicular disease in sows and an increased neonatal mortality (SVA/KS/2018). Again, this isolate was propagated on ST cells. The isolate was passaged 3 times in cell culture and was diluted with MEM into two stocks for inoculation: $1x10^7$ $TCID_{50}$/mL and $1x10^5$ $TCID_{50}$/mL.

Animal studies

Study 1: Forty-six pigs of mixed sex were farrowed on-site at the National Animal Disease Center (NADC) in Ames, IA and housed under ABSL-2 conditions for the experiment. At weaning, pigs were randomly ear tagged and assigned to 4 treatment groups: control (n=8), vaccinated (vax) (n=8), challenge (n=15), and vax+challenge (n=15). At three weeks of age, the vax and vax+challenge treatment groups were immunized by injection intramuscularly (20g-1in needle) in the right side of the neck with 2 mL of inactivated SVA (SVA/IA/2015) plus adjuvant. A second dose was given intramuscularly on the left side of the neck (2 mL) three weeks later. At 0 days post challenge (dpc), two weeks after boost, animals in the challenge and vax+challenge groups were intranasally challenged with 5 mL of SVA/SD/2015 ($1x10^7$ $TCID_{50}$/mL) divided between both nostrils (Table 1). Pigs were bled on -35, -14, 0, 3, 5, 7, 14 and 21 dpc. Group oral fluids and individual rectal swabs were collected daily from 0-10 dpc and on 14 and 21 dpc from challenged animals. Animals were euthanized on 21 dpc.

Study 2: Twenty sows were purchased from a commercial source in the Midwest of the United States and transported to the NADC. All animals were clinically free of lesions at the start of the study. Sows were bred via artificial insemination and 18/20 sows were confirmed pregnant by ultrasound. The two open sows were placed in the vax+challenge group while the remaining sows were randomly assigned to 4 groups: control (n=3), vax (n=3), challenge (n=5), and vax+challenge (n=9). At -35 dpc (~70 days of gestation) sows in the vax and vax+challenge treatment groups were immunized intramuscularly in the right side of the neck with 2 mL of inactivated SVA (SVA/IA/2015) plus adjuvant. A second dose was given intramuscularly on the left side of the neck (2 mL) three weeks later. At 0 dpc (~104 days of gestation) animals were separated into ABSL-2 rooms based on treatment and intranasally challenged with 5 mL of SVA/KS/2018 ($1x10^7$ $TCID_{50}$/mL) split between both nostrils (Table 1). Challenged sows were bled on -35, -14, 0, 3, 7, and 14 dpc and rectal swabbed and observed for vesicular lesions on 0-3, 5, 7, and 14 dpc. Sows were euthanized between 28 and 30 dpc.

Study 3: Piglets born to control sows (n=3 sows, 39 piglets) and vax sows (n=3 sows, 23 piglets) from Study 2 were challenged orally with 2 mL of SVA/KS/18 ($1x10^5$ $TCID_{50}$/mL) while still suckling their dams (3-6 days of age) (Table 1). Piglets were ear notched for identification. They were bled on 0, 5, and 14 days post inoculation (dpi) and rectal swabbed on 0, 1, 2, and 5 dpi. In addition, piglets were observed daily for clinical signs including lethargy, diarrhea and neurologic signs. All mortalities or piglets euthanized were recorded.

Vesicular lesions were scored with a 5-point system: each foot that displayed a vesicular lesion was given 1 point and a vesicle on the snout was assigned 1 point. An animal was given a score of zero if no lesions were identified. Scores were assigned on 0, 3, 5, 7, and 9 dpc. Total clinical score was calculated by adding up the scores for all pigs in the group. Finally, pigs were observed daily for clinical signs of lameness, lethargy, and inappetence.

Blood was collected in serum separator tubes (BD Vacutainer®, Franklin Lakes, NJ) and centrifuged to harvest serum. Fecal swabs were collected using a sterile polyester tipped applicator (Puritan Medical Products, Guilford, ME), immersed in 3 mL of serum-free MEM. Oral fluids were collected with a white cotton rope hung for a half hour at pig shoulder height in each pen containing all pigs in the treatment group. Liquid was manually squeezed from the rope into a plastic bag and poured into vials for storage. All samples were stored at -80°C for future testing.

SVA nucleic acid quantification

Serum, swabs, and oral fluid samples were extracted and tested by real time RT-qPCR as previously described [23]. Briefly, RNA was extracted from samples using the MagMAX™ Pathogen RNA/DNA kit (Applied Biosystems). Next, 5 µL of extracted product was added to 20 µL of the Path-ID™ Multiplex One-Step RT-PCR reaction master mix (Applied Biosystems) for fecal swabs and oral fluids or 20 µL AgPath-ID™ One step RT-PCR master mix (Applied Biosystems) for sera and vesicle swabs. The forward primer sequence was 5'-TGCCTTGGATACTGCCTGATAG-3', the reverse primer sequence was 5'-GGTGCCAGAGGCTGTATCG-3' and the probe sequence was 5'-CGACGGCCTAGTCGGTCGGTT-3'. The primers and probe were designed to target a conserved region between the 5' untranslated region (5'UTR) and protein L containing nucleotides 602-710 of the SVA genome. RNA copies were calculated based on a standard curve generated from a plasmid containing the target region, and C_t values greater than 35 were considered negative.

Virus neutralization (VN) assay

The virus neutralization assay has previously been described [23]. In brief, serum samples were heat-inactivated at 56 °C for 30 min, serially diluted 1:4 (up to 1:4096) in MEM and repeated in quadruplicate. An equal volume of diluted challenge virus, SVA/SD/15 or SVA/KS/18, (~200 $TCID_{50}$) was added to the serum and incubated for 1 hour. The virus-serum mixture was transferred to 96-well plates of ST cells. Plates were microscopically evaluated for cytopathic effect (CPE) daily for 4 days. Titers were recorded as the reciprocal of the highest dilution of serum at which the infectivity of the SVA isolate was completely neutralized in 50% of the inoculated wells. Virus neutralization titers of ≤16 were considered negative.

Statistical analysis

Statistical analysis was performed by multiple t-tests without the assumption of consistent standard deviation (SD). Multiple comparisons were corrected using the Holm-Sidak method with alpha =0.5. Statistical analysis and graphs were performed and prepared using GraphPad Prism Version 8.1.2 (332) software.

Results

Study 1

Two groups were sham challenged. The control pigs did not develop clinical signs of vesicular disease, SVA RNA was not detected in any sample tested by PCR, and pigs did not seroconvert with neutralizing antibody titers of ≤16 at 21 dpc. In the vax group, no pigs were observed with vesicular lesions and SVA RNA was not detected in tested serum, rectal swabs, or oral fluids after sham challenge. After the first dose of vaccine, 3/8 pigs had titers greater than 16. Following two doses of vaccine, all pigs (8/8) in the vax group developed a VN titer of 256 (21 dpc) (Figure 1A).

Two groups were intranasally challenged with SVA/SD/2015. In the challenge group, 12/15 pigs developed vesicular lesions after challenge (Figure 2A). Lesions were first observed on 3 dpc with vesicle development on the coronary bands of 2 pigs and the peak number of pigs observed with lesions occurred on 7 dpc. Snout lesions were not observed until 5 dpc with a total of 5/15 pigs eventually developing erosions on the snout. The vax+challenge group did not develop any signs of vesicular disease following challenge.

SVA RNA was detected in rectal swabs by 1 dpc in both the challenge (12/15) and vax+challenge (13/15) pigs (Figure 2B). However, all pigs in the vax+challenge group were negative by 2 dpc and remained negative for SVA by PCR in rectal swabs until the end of the study. In contrast, SVA RNA was detected in rectal swabs from the challenge group from 1 dpc until the end of study (21 dpc) with 9/15 (60%) of pigs with a positive rectal swab at necropsy. There was a decrease in SVA detection in this group between 8 and 10 dpc, but genomic copies increased again by 14 dpc. SVA RNA was detected in serum in most pigs (13/15) in the challenge group during the first week after challenge, with two pigs that did not develop a measurable viremia (Figure 2C). No virus was detected in serum from the vax+challenge group. Similar to the serum PCR results, oral fluids demonstrated viral detection in the challenge group, but not in the vax+challenge group (Figure 2D). Viral levels in the oral fluids remained similar throughout the study.

All pigs in the challenge group started with a VN titer ≤16 prior to challenge (0 dpc) and at 21 dpc VN titers ranged from 64 to 256 (Figure 1A), except one pig with a titer of 16. This pig was one of the two animals in the challenge group without a detectable viremia; although, it did have positive rectal swabs throughout the study. Similar to the vax group, 7/15 pigs in the vax+challenge group had VN titers >16 after the first dose of vaccine. Prior to challenge, all pigs in the vax+challenge group but one had titers >16 following two doses of vaccine (0 dpc). At the end of the study (21 dpc), titers in the vax+challenge group ranged from 64 to 1024 which was similar to those pigs in the challenge group (Figure 1A).

Study 2

Two groups of sows were sham challenged. Control sows did not develop clinical signs of vesicular disease, SVA RNA was not detected in any sample tested by PCR, and sows did not develop a neutralizing antibody response with titers ≤ 16 at the end of the study. In the vax group, no sows were observed with vesicular lesions and SVA RNA was not detected in tested serum or rectal swabs after sham challenge. Animals in the vax group had VN titers ranging from 64 to 256 following one dose of vaccine (-14 dpc) and 256 to 1024 after two doses of vaccine (0 dpc) (Figure 1B).

Two groups were intranasally challenged with SVA/KS/18. In the challenge group, 2/5 sows developed vesicular lesions on their snouts (Figure 3A). No lesions were observed on coronary bands. The vax+challenge group did not develop any signs of vesicular disease following challenge. SVA RNA was first detected in two rectal swabs from both the challenge group (2/5) and the vax+challenge (2/9) group on 5 dpc (Figure 3B). The remaining rectal swabs were negative by PCR. SVA RNA was detected in the serum of two animals in the challenge group on 3 dpc (Figure 3C). In contrast, SVA was not detected in the serum from any animals in the vax+challenge group. In general, low levels of SVA were detected by PCR after challenge and there was no significant difference between the two groups in rectal swabs or serum. In total, SVA RNA was detected by PCR in one sample, either serum, rectal swab, or vesicular swab in 4/5 animals in the challenge group and only 2/9 animals in the vax+challenge group.

All sows started with a VN titer ≤ 16 prior to challenge (0 dpc). VN titers ranged from 1024 to 4096 in the challenge group at necropsy (Figure 1B). The vax+challenge group had VN titers ranging between 16 and 64 after one dose and from 64 to 1024 following two doses of vaccine (0 dpc). At the end of study, titers in the vax+challenge group remained between 64 and 1024. There was no significant difference in titers between the challenged and vaccinated animals at necropsy. Although animals in the challenge group had limited amounts of shedding detected by PCR, all seroconverted after challenge and developed neutralizing antibody titers.

Study 3

Control sows (n=3) and vax sows (n=3) farrowed and all piglets were challenged orally with SVA/KS/2018 around 3-6 days of age, while remaining suckling the sow. No clinical signs were observed in any of the piglets, except 2-3 piglets with a mild transient diarrhea in litters born to both control sows and vax sows. Two piglets were euthanized from the control sow litters on 10 dpi due to failure to thrive, however no piglets in the vax sow litters had to be euthanized (Table 2). Pre-weaning mortality after challenge in the control sow litters was 5% and 0% in the vax sow litters. All piglets born to control sows were positive for SVA by PCR in serum samples collected on 5 dpi (39/39) (Figure 4A). A few piglets had positive 1 and 2 dpi rectal swabs; however, by 5 dpi 29/39 piglets had a PCR positive rectal swab (Figure 4B). Piglets born from vaccinated sows did not have any PCR positive serum or rectal swabs after challenge (0/23).

The first four ear notched piglets were selected from each litter for VN testing on 0 and 14 dpi serum (Figure 1C). Prior to challenge, piglets tested from control sows had neutralizing titers ≤ 16. After challenge (14 dpi) piglets had neutralizing titers from 256 to 4096. In contrast, piglets born to vaccinated sows had neutralizing titers ranging from 256 to 1024 prior to challenge. After challenge, VN titers decreased in most piglets born to vaccinated sows and ranged from 16 to 1024.

Discussion

Vaccines play a critical role in the prevention and control of viral and bacterial diseases of swine [24]. Inactivated whole-virus vaccines do not replicate in the host; therefore, they do not cause clinical disease and there is no potential of reversion to virulence like live-attenuated vaccines. In this study, a whole-virus SVA inactivated vaccine was given in two doses to weaned pigs and sows and its efficacy against SVA challenge was evaluated. In addition, maternal immunity provided by vaccinated sows was assessed by challenging suckling piglets.

Weaned pigs have previously been utilized for a SVA vaccine efficacy study and animals given two doses of an inactivated SVA vaccine and challenged with a heterologous SVA isolate developed clinical signs and shed virus [19]. In contrast, vaccinated pigs in study 1 after homologous challenge did not develop clinical disease and viral nucleic acid was only detected in rectal swabs on 1 dpc. It is likely the viral nucleic acid detected was pass through from the intranasal inoculation, but minor replication within the pig cannot be ruled out. Although both studies used animals close in age and a similar virus used to make the inactivated vaccine, there were a number of differences between the studies that could have contributed to the different outcome observed including: quantity of antigen in the vaccine, inactivation process, adjuvant composition, homology of challenge isolate to the vaccine strain, and challenge dose.

Other previously published studies have tested the efficacy of an inactivated SVA vaccine but utilized growing pigs rather than weaned pigs and challenged with homologous strains [20, 21]. In one study pigs only received one dose of inactivated SVA vaccine prior to challenge and pigs were protected from the development of clinical disease, but further samples such as rectal/nasal swabs were not collected to look for viral shedding [20]. The virus used for vaccination was the same virus used to challenge the pigs thus more likely to be effective than versus a heterologous challenge. After two doses, vaccinated animals in both study 1 and study 2 had similar VN titers to non-vaccinated challenged animals. Further work would need to be performed to evaluate if one dose would have been sufficient to provide protection.

Two pigs in the challenge group of study 1 did not develop a detectable viremia; although, both animals did have multiple PCR positive rectal swabs and they both developed vesicular lesions. Of the two animals that did not test PCR positive for SVA in the serum, one did not develop a neutralizing antibody response, while the other had a neutralizing antibody titer of 256. Three other animals that did not develop vesicular lesions tested PCR positive in all serum and rectal swabs collected through 6 dpi. Despite this study and the work of others, there are still questions surrounding the pathogenesis of SVA including what drives the development of neutralizing antibody titers and why some animals do not develop vesicular lesions.

Based on the success in study 1, study 2 was performed with sows since they may be the more likely candidate for vaccination. The cull sow network, which can include multiple collection points and trucking over large distances to group sows for slaughter plants, has been recognized as a disease transmission risk [25]. In the US, sows with vesicular lesions have been observed on a regular basis when arriving at sow slaughter plants (J. Korslund, personal communication), thus vaccinated sows prior to entering the cull sow market could reduce or prevent the number of foreign animal disease (FAD) investigations occurring at sow slaughter plants. In 2019 there were 2,135 FAD investigations in the US with 1,845 involving vesicular disease conditions in pigs [26]. These investigations are costly in terms of both time and resources to rule out FMDV every time a vesicle is observed on a pig.

This was the first time SVA/KS/18 was used to challenge mature swine after isolation. This isolate was selected as it was isolated from an SVA outbreak on a sow farm with increased neonatal mortality. Based on results of the challenged sows, there was very little detectable virus in either the serum or swabs samples collected, which makes it difficult to demonstrate reduction of viral replication and shedding between the challenge group and the vax+challenge group.

Evidence that the challenge virus was recognized despite limited PCR detection was the robust neutralizing antibody response generated by animals in the challenge group. In addition, 2/5 challenge animals did develop vesicles on their snouts. Experimental SVA challenge work with sows is limited, but another study using sows reported 4/5 sows developed vesicular lesions after challenge and field observations at sow farms experiencing SVA outbreaks have reported a large range in the percentage of sows observed with vesicular lesions [8, 27, 28]. Thus, it could be expected that not all sows develop lesions after challenge. Overall, the lack of lesion development in all nine animals of the vax+challenge group compared to lesion development in 2/5 challenged animals provides evidence that the vaccine protects against the development of vesicular disease in mature animals. Further evaluation of this vaccine in mature animals should be performed with another challenge strain to evaluate difference in viral replication and shedding.

Study 3 evaluated if the inactivated SVA vaccine given to sows could provide protection to nursing piglets challenged with SVA. In the field, there have been multiple reports of increased neonatal mortality in piglets less than 1 week of age that tested positive for SVA [8, 29, 30]. SVA positive piglets have been reported with clinical signs including lethargy, diarrhea, and neurologic signs [31]. Piglets challenged between 3-6 days of age after suckling control sows did not develop any clinical signs beyond a mild transient diarrhea in a couple of piglets, which was also observed in a few piglets suckling vaccinated sows. The pre-weaning mortality observed in this study after challenge was 5% for control sow litters and 0% for vax sow litters, while overall pre-weaning mortality was 10% and 4% respectively. The mean piglet pre-weaning mortality rate in commercial swine herds ranges between 10% and 20% [32] and the pre-weaning mortality during SVA outbreaks in the field have been reported to be up to 60% [28].

Although the control litters had a higher percentage of pre-weaning mortality compared to the vax litters, it still falls within the commercial norm. In addition, the larger litter sizes in the control sow litters (n=14, 14, 13) versus the vax sow litters (n=9, 9, 6) may have contributed to the higher pre-weaning mortality in this study.

Piglets born to vaccinated sows had VN titers measured in serum prior to challenge demonstrating that sows vaccinated around 70 days of gestation and boosted around 90 days of gestation transferred immunity to their piglets. After challenge, VN titers decreased in those piglets, which follows waning maternal immunity. A previous report also described the transfer of maternal immunity to suckling pigs, but did not challenge piglets to determine if those titers were protective [33]. Negative PCR results showed that maternal immunity from vaccinated sows was able to protect piglets less than one week-of-age from SVA challenge. In contrast, piglets that did not suckle immunized dams replicated virus and may be an important source of viral amplification during a SVA outbreak at a naïve farrowing facility.

In the US when a vesicle is observed on a pig, animal movement is stopped while trained personnel initiate a FAD investigation to rule out FMD. These FAD investigations and sample testing are costly to both the industry and the government, especially if SVA continues to remain endemic in the US swine herd. In addition, with the consistent occurrence of vesicular lesions in swine, veterinarians and producers in the swine industry may become complacent in assuming lesions are due to SVA infection. If FMDV were to enter the country and remain undetected for a period of time, the consequences could be devastating for not only the swine industry, but also animal agriculture and the economy. An effective SVA vaccine could be a valuable tool for the swine industry that could have a positive impact on welfare and reduce the economic burden of investigating vesicular disease cases.

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Conflict of interest

The authors declare that they have no conflict of interests regarding the publication of this study

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References

1. Hales LM, Knowles NJ, Reddy PS, Xu L, Hay C, Hallenbeck PL. Complete genome sequence analysis of Seneca Valley virus-001, a novel oncolytic picornavirus. The Journal of general virology. 2008;89(Pt 5):1265-75. doi: 10.1099/vir.0.83570-0. PubMed PMID: 18420805.
2. Fan H, Zhu H, Li S, Shi M, Zhou E, Wang X, et al. Identification of linear B cell epitopes on VP1 and VP2 proteins of Senecavirus A (SVA) using monoclonal antibodies. Veterinary

microbiology. 2020;247:108753. Epub 2020/08/10. doi: 10.1016/j.vetmic.2020.108753. PubMed PMID: 32768207.

3. Maggioli MF, Lawson S, de Lima M, Joshi LR, Faccin TC, Bauermann FV, et al. Adaptive Immune Responses following Senecavirus A Infection in Pigs. Journal of virology. 2018;92(3). Epub 2017/11/17. doi: 10.1128/JVI.01717-17. PubMed PMID: 29142122; PubMed Central PMCID: PMCPMC5774895.

4. Vannucci FA, Linhares DC, Barcellos DE, Lam HC, Collins J, Marthaler D. Identification and Complete Genome of Seneca Valley Virus in Vesicular Fluid and Sera of Pigs Affected with Idiopathic Vesicular Disease, Brazil. Transboundary and emerging diseases. 2015;62(6):589-93. doi: 10.1111/tbed.12410. PubMed PMID: 26347296.

5. Leme RA, Zotti E, Alcantara BK, Oliveira MV, Freitas LA, Alfieri AF, et al. Senecavirus A: An Emerging Vesicular Infection in Brazilian Pig Herds. Transboundary and emerging diseases. 2015;62(6):603-11. doi: 10.1111/tbed.12430. PubMed PMID: 26398783.

6. Guo B, Pineyro PE, Rademacher CJ, Zheng Y, Li G, Yuan J, et al. Novel Senecavirus A in Swine with Vesicular Disease, United States, July 2015. Emerging infectious diseases. 2016;22(7):1325-7. doi: 10.3201/eid2207.151758. PubMed PMID: 27314645; PubMed Central PMCID: PMC4918180.

7. Hause BM, Myers O, Duff J, Hesse RA. Senecavirus A in Pigs, United States, 2015. Emerging infectious diseases. 2016;22(7):1323-5. doi: 10.3201/eid2207.151591. PubMed PMID: 27314580; PubMed Central PMCID: PMC4918151.

8. Canning P, Canon A, Bates JL, Gerardy K, Linhares DC, Pineyro PE, et al. Neonatal Mortality, Vesicular Lesions and Lameness Associated with Senecavirus A in a U.S. Sow Farm. Transboundary and emerging diseases. 2016;63(4):373-8. doi: 10.1111/tbed.12516. PubMed PMID: 27213868.

9. Montiel N, Buckley A, Guo B, Kulshreshtha V, VanGeelen A, Hoang H, et al. Vesicular Disease in 9-Week-Old Pigs Experimentally Infected with Senecavirus A. Emerging infectious diseases. 2016;22(7):1246-8. doi: 10.3201/eid2207.151863. PubMed PMID: 27315363; PubMed Central PMCID: PMC4918149.

10. Joshi LR, Fernandes MH, Clement T, Lawson S, Pillatzki A, Resende TP, et al. Pathogenesis of Senecavirus A infection in finishing pigs. The Journal of general virology. 2016;97(12):3267-79. doi: 10.1099/jgv.0.000631. PubMed PMID: 27902357.

11. Chen Z, Yuan F, Li Y, Shang P, Schroeder R, Lechtenberg K, et al. Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A. Virology. 2016;497:111-24. doi: 10.1016/j.virol.2016.07.003. PubMed PMID: 27459668.

12. Xu W, Hole K, Goolia M, Pickering B, Salo T, Lung O, et al. Genome wide analysis of the evolution of Senecavirus A from swine clinical material and assembly yard environmental samples. PloS one. 2017;12(5):e0176964. Epub 2017/05/06. doi: 10.1371/journal.pone.0176964. PubMed PMID: 28475630; PubMed Central PMCID: PMCPMC5419577.

13. Wu Q, Zhao X, Bai Y, Sun B, Xie Q, Ma J. The First Identification and Complete Genome of Senecavirus A Affecting Pig with Idiopathic Vesicular Disease in China. Transboundary and emerging diseases. 2016. doi: 10.1111/tbed.12557. PubMed PMID: 27539949.

14. Sun D, Vannucci F, Knutson TP, Corzo C, Marthaler DG. Emergence and whole-genome sequence of Senecavirus A in Colombia. Transboundary and emerging diseases. 2017. doi: 10.1111/tbed.12669. PubMed PMID: 28714178.

15. Saeng-Chuto K, Rodtian P, Temeeyasen G, Wegner M, Nilubol D. The first detection of Senecavirus A in pigs in Thailand, 2016. Transboundary and emerging diseases. 2017. doi: 10.1111/tbed.12654. PubMed PMID: 28474854.

16. Arzt J, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, et al. First Detection and Genome Sequence of Senecavirus A in Vietnam. Microbiol Resour Announc. 2019;8(3). Epub 2019/01/29. doi: 10.1128/MRA.01247-18. PubMed PMID: 30687818; PubMed Central PMCID: PMCPMC6346150.

17. McElwain TF, Thumbi SM. Animal pathogens and their impact on animal health, the economy, food security, food safety and public health. Rev Sci Tech. 2017;36(2):423-33. Epub 2018/08/29. doi: 10.20506/rst.36.2.2663. PubMed PMID: 30152474; PubMed Central PMCID: PMCPMC6561776.

18. Knight-Jones TJ, Rushton J. The economic impacts of foot and mouth disease - what are they, how big are they and where do they occur? Prev Vet Med. 2013;112(3-4):161-73. Epub 2013/08/21. doi: 10.1016/j.prevetmed.2013.07.013. PubMed PMID: 23958457; PubMed Central PMCID: PMCPMC3989032.

19. Sharma B, Fernandes MHV, de Lima M, Joshi LR, Lawson S, Diel DG. A Novel Live Attenuated Vaccine Candidate Protects Against Heterologous Senecavirus A Challenge. Front Immunol. 2019;10:2660. Epub 2019/12/19. doi: 10.3389/fimmu.2019.02660. PubMed PMID: 31849928; PubMed Central PMCID: PMCPMC6901945.

20. Yang F, Zhu Z, Cao W, Liu H, Zhang K, Tian H, et al. Immunogenicity and protective efficacy of an inactivated cell culture-derived Seneca Valley virus vaccine in pigs. Vaccine. 2018;36(6):841-6. Epub 2018/01/10. doi: 10.1016/j.vaccine.2017.12.055. PubMed PMID: 29310900.

21. Li N, Qiao QL, Guo HF, Wang BY, Huang Q, Wang Z, et al. Evaluation of immunogenicity and protective efficacy of a novel Senecavirus A strain-based inactivated vaccine in mice. Res Vet Sci. 2021;142:133-40. Epub 2021/12/25. doi: 10.1016/j.rvsc.2021.12.010. PubMed PMID: 34952258.

22. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. The American Journal of Hygiene. 1938;27(3):493-7.

23. Buckley A, Montiel N, Guo B, Kulshreshtha V, van Geelen A, Hoang H, et al. Dexamethasone treatment did not exacerbate Seneca Valley virus infection in nursery-age pigs. BMC Vet Res. 2018;14(1):352. Epub 2018/11/21. doi: 10.1186/s12917-018-1693-8. PubMed PMID: 30453952; PubMed Central PMCID: PMCPMC6245856.

24. Dhakal S, Renukaradhya GJ. Nanoparticle-based vaccine development and evaluation against viral infections in pigs. Vet Res. 2019;50(1):90. Epub 2019/11/07. doi: 10.1186/s13567-019-0712-5. PubMed PMID: 31694705; PubMed Central PMCID: PMCPMC6833244.

25. Blair B, Lowe J. Describing the cull sow market network in the US: A pilot project. Prev Vet Med. 2019;162:107-9. Epub 2019/01/10. doi: 10.1016/j.prevetmed.2018.11.005. PubMed PMID: 30621887.

26. USDA, APHIS, VS. Calendar Year 2019 Update: FAD Investigation Report. National Preparedness and Incident Coordination (NPIC). 2020.

27. Maggioli MF, Fernandes MHV, Joshi LR, Sharma B, Tweet MM, Noll JCG, et al. Persistent Infection and Transmission of Senecavirus A from Carrier Sows to Contact Piglets. Journal of virology. 2019;93(21). Epub 2019/08/23. doi: 10.1128/JVI.00819-19. PubMed PMID: 31434730; PubMed Central PMCID: PMCPMC6803263.
28. Segales J, Barcellos D, Alfieri A, Burrough E, Marthaler D. Senecavirus A: An Emerging Pathogen Causing Vesicular Disease and Mortality in Pigs? Veterinary pathology. 2016. doi: 10.1177/0300985816653990. PubMed PMID: 27371541.
29. Leme RA, Oliveira TE, Alcantara BK, Headley SA, Alfieri AF, Yang M, et al. Clinical Manifestations of Senecavirus A Infection in Neonatal Pigs, Brazil, 2015. Emerging infectious diseases. 2016;22(7):1238-41. doi: 10.3201/eid2207.151583. PubMed PMID: 27315157; PubMed Central PMCID: PMC4918167.
30. Gimenez-Lirola LG, Rademacher C, Linhares D, Harmon K, Rotolo M, Sun Y, et al. Serological and Molecular detection of Senecavirus A associated with an outbreak of Swine Idiopathic Vesicular Disease and Neonatal Mortality. Journal of clinical microbiology. 2016. doi: 10.1128/JCM.00710-16. PubMed PMID: 27225408.
31. Leme RA, Oliveira TE, Alfieri AF, Headley SA, Alfieri AA. Pathological, Immunohistochemical and Molecular Findings Associated with Senecavirus A-Induced Lesions in Neonatal Piglets. Journal of comparative pathology. 2016. doi: 10.1016/j.jcpa.2016.06.011. PubMed PMID: 27473601.
32. Muns R, Nuntapaitoon P, Tummaruk P. Non-infectious causes of pre-weaning mortality in piglets Livestock Science. 2016;184:46-57. doi: doi.org/10.1016/j.livsci.2015.11.025.
33. Yang F, Zhu Z, Liu H, Cao W, Zhang W, Wei T, et al. Evaluation of Antibody Response in Sows after Vaccination with Senecavirus A Vaccine and the Effect of Maternal Antibody Transfer on Antibody Dynamics in Offspring. Vaccines (Basel). 2021;9(10). Epub 2021/10/27. doi: 10.3390/vaccines9101066. PubMed PMID: 34696174; PubMed Central PMCID: PMCPMC8538203.

Figures

Figure 1: Virus neutralization titers. VN titers from serum collected in Study 1 against SVA/IA/2015 (A), and against SVA/KS/2018 in Study 2 (B) and Study 3 (C). The mean titer for the challenge group is represented by red bars, the vax+challenge group by blue bars, the vax group by purple bars, and the control group by green bars (A and B). Mean titers for piglets born to control sows is colored green and data from piglets born to vaccinated sows is colored purple (C). Error bars represent SD.

Figure 2: Clinical scores and viral load as determined by RT-qPCR in Study 1. A) Ruptured coronary band lesion observed in a challenged pig and a snout erosion observed on a challenge pig on 7 dpi. Animals were scored on a scale of 0-5. Scores were added up for each group and the total clinical score plotted. The mean $\log_{10}$(genomic copies/mL) for challenge pigs and vax+challenge pigs in rectal swabs (B) and serum (C). Error bars represent SD (**P < 0.01; ***P < 0.001; ****P < 0.0001). The $\log_{10}$(genomic copies/mL) of oral fluids collected from each group. Challenge pig data colored red and vax+challenge pig data colored blue.

Figure 3: Clinical scores and viral load as determined by RT-qPCR in Study 2. A) Intact vesicle on the snout of a challenged sow at 8 dpi and the same lesion ruptured and healing on 14 dpi. B) Animals were scored on a scale of 0-5. Scores were added up for each group and the total clinical score plotted. The mean $\log_{10}$ (genomic copies/mL) for challenge pigs and vax+challenge pigs in rectal swabs (C) and serum (D). Error bars represent SD. Challenged pig data colored red and vax+challenge pig data colored blue.

Figure 4: Viral load as determined by RT-qPCR in Study 3. The mean $\log_{10}$(genomic copies/mL) for control sow piglets and vax sow piglets in rectal swabs (A) and serum (B). Error bars represent SD (****P < 0.0001). Control sow piglet data colored green and vax sow piglet data colored purple.

Table 1: Experimental design of animal studies

Study	Age at challenge	Group	N	Vaccine[a]	Timing	Challenge	Dose
Study 1	8 weeks	Control	8	None		Sham	
		Vax	8	SVA/IA/2015	-35 and -14 dpc	Sham	
		Challenge	15	None		SVA/SD/2015	$5x10^{7}$ $TCID_{50}$
		Vax+challenge	15	SVA/IA/2015	-35 and -14 dpc	SVA/SD/2015	$5x10^{7}$ $TCID_{50}$
Study 2	Mature sows	Control	3	None		Sham	
		Vax	3	SVA/IA/2015	-35 and -14 dpc	Sham	
		Challenge	5	None		SVA/KS/2018	$5x10^{7}$ $TCID_{50}$
		Vax+challenge	9	SVA/IA/2015	-35 and -14 dpc	SVA/KS/2018	$5x10^{7}$ $TCID_{50}$
Study 3	3-6 days	Control sow litters (n=12, 13, 14)	3			SVA/KS/2018	$2x10^{5}$ $TCID_{50}$
		Vax sow litters (n=6, 8, 9)	3			SVA/KS/2018	$2x10^{5}$ $TCID_{50}$

[a] Combined with an oil-in-water adjuvant at 12.5%

Table 2: Piglet mortality in Study 3

Group	Born alive	Stillborn/mummies	Mortality pre-challenge	Piglets challenged	Age at challenge (days)	Mortality post-challenge
Control sow litters	13	1	1[a]	12	3	0
	14	2	1[b]	13	6	0
	14	0	0	14	6	2[b]
Vax sow litters	6	0	0	6	6	0
	9	1	1[a]	8	4	0
	9	0	0	9	4	0

[a] Found dead
[b] Euthanized due to failure to thrive

Figures

Figure 1: Virus neutralization titers. VN titers from serum collected in Study 1 against SVA/IA/2015 (A), and against SVA/KS/2018 in Study 2 (B) and Study 3 (C). The mean titer for the challenge group is represented by red bars, the vax+challenge group by blue bars, the vax group by purple bars, and the control group by green bars (A and B). Mean titers for piglets born to control sows is colored green and data from piglets born to vaccinated sows is colored purple (C). Error bars represent SD.

Figure 2: Clinical scores and viral load as determined by RT-qPCR in Study 1. A) Ruptured coronary band lesion observed in a challenged pig and a snout erosion observed on a challenge pig on 7 dpi. Animals were scored on a scale of 0-5. Scores were added up for each group and the total clinical score plotted. The mean $\log_{10}$(genomic copies/mL) for challenge pigs and vax+challenge pigs in rectal swabs (B) and serum (C). Error bars represent SD (**P < 0.01; ***P < 0.001; ****P < 0.0001). The $\log_{10}$(genomic copies/mL) of oral fluids collected from each group. Challenge pig data colored red and vax+challenge pig data colored blue.

Figure 3: Clinical scores and viral load as determined by RT-qPCR in Study 2. A) Intact vesicle on the snout of a challenged sow at 8 dpi and the same lesion ruptured and healing on 14 dpi. B) Animals were scored on a scale of 0-5. Scores were added up for each group and the total clinical score plotted. The mean $\log_{10}$ (genomic copies/mL) for challenge pigs and vax+challenge pigs in rectal swabs (C) and serum (D). Error bars represent SD. Challenged pig data colored red and vax+challenge pig data colored blue.

Figure 4: Viral load as determined by RT-qPCR in Study 3. The mean $\log_{10}$(genomic copies/mL) for control sow piglets and vax sow piglets in rectal swabs (A) and serum (B). Error bars represent SD (****P < 0.0001). Control sow piglet data colored green and vax sow piglet data colored purple.

Figure 1

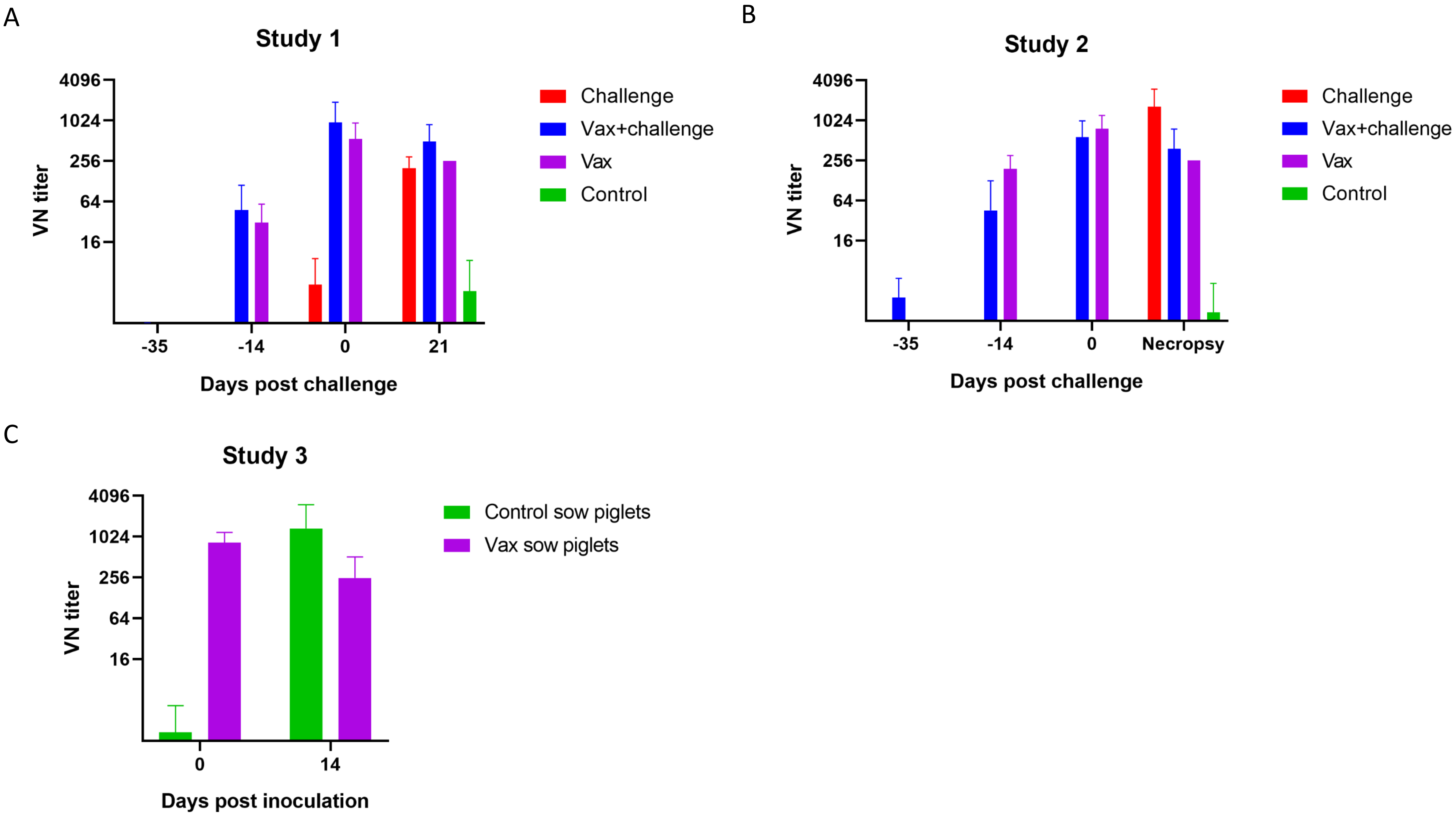

A
Study 1
4096
1024
256
64
16
VN titer
-35
-14
0
21
Days post challenge
Challenge
Vax+challenge
Vax
Control
B
Study 2
4096
1024
256
64
16
VN titer
-35
-14
0
Necropsy
Days post challenge
Challenge
Vax+challenge
Vax
Control
C
Study 3
4096
1024
256
64
16
VN titer
0
14
Days post inoculation
Control sow piglets
Vax sow piglets

Figure 2

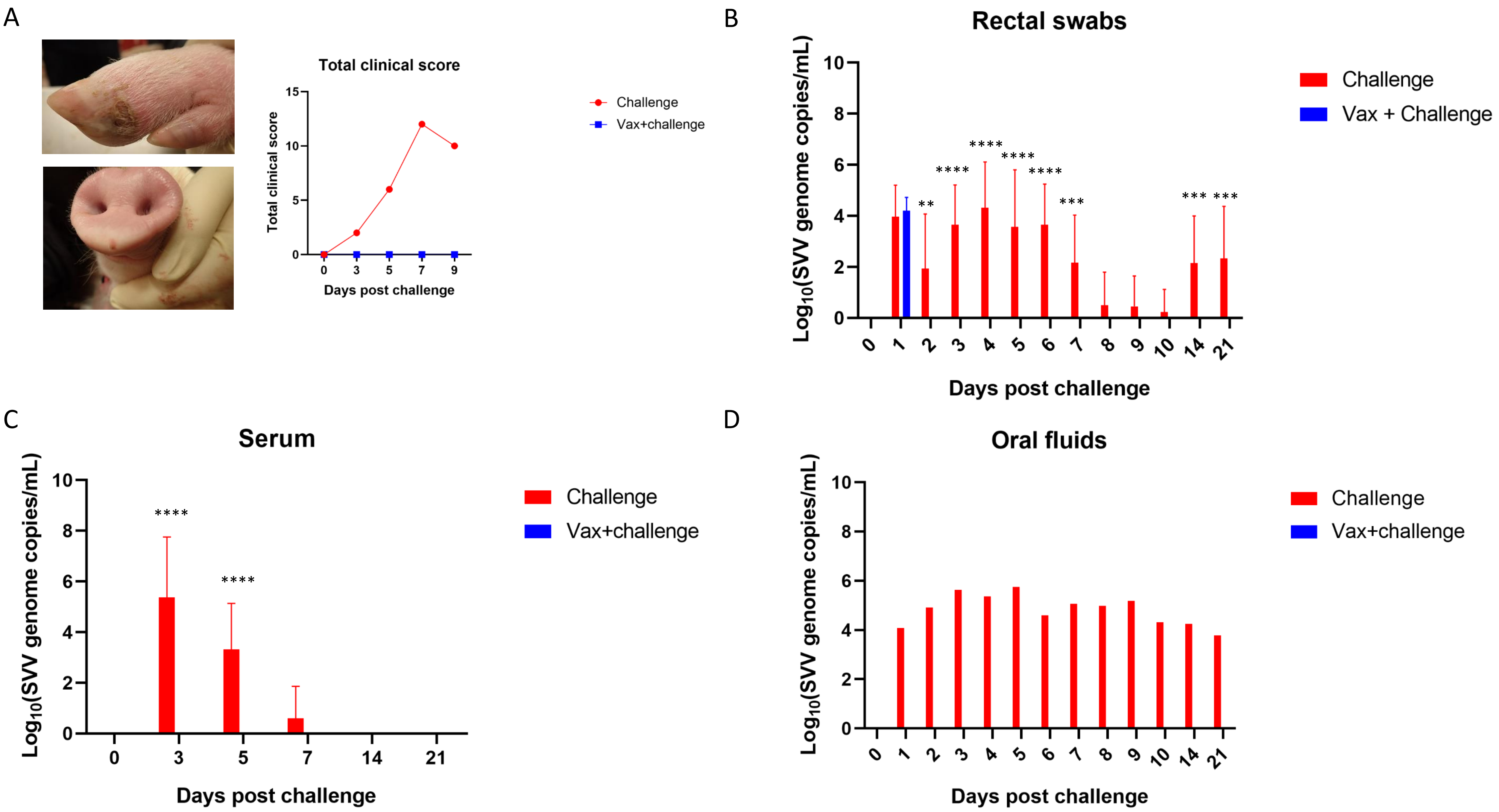

A
Total clinical score
Total clinical score
0
5
10
15
0
3
5
7
9
Days post challenge
Challenge
Vax+challenge
B
Rectal swabs
Log_{10}(SVV genome copies/mL)
0
2
4
6
8
10
**
****
****
****
****
***
*** ***
0
1
2
3
4
5
6
7
8
9
10
14
21
Days post challenge
Challenge
Vax + Challenge
C
Serum
Log_{10}(SVV genome copies/mL)
0
2
4
6
8
10
****
****
0
3
5
7
14
21
Days post challenge
Challenge
Vax+challenge
D
Oral fluids
Log_{10}(SVV genome copies/mL)
0
2
4
6
8
10
0
1
2
3
4
5
6
7
8
9
10
14
21
Days post challenge
Challenge
Vax+challenge

Figure 3

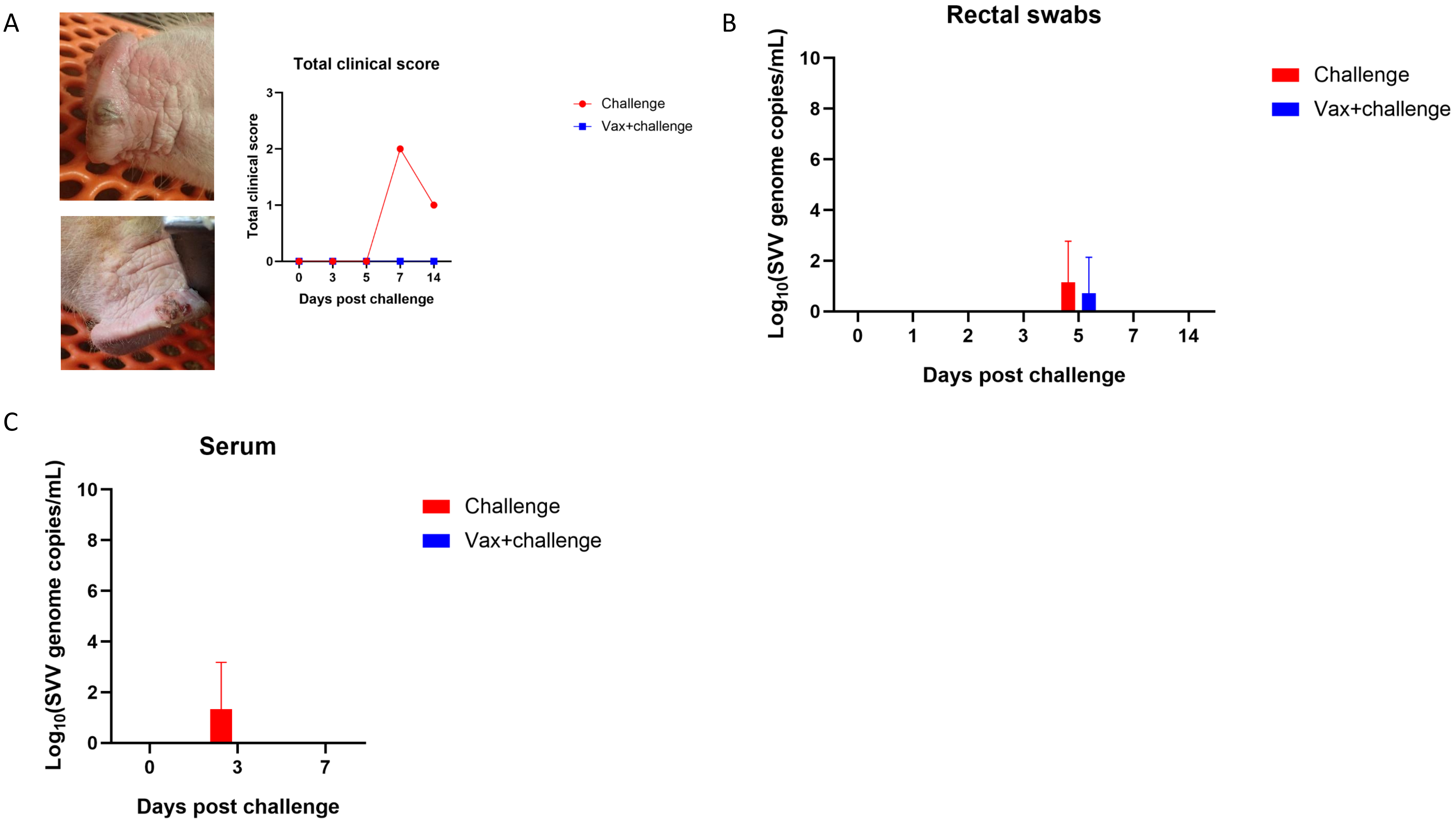
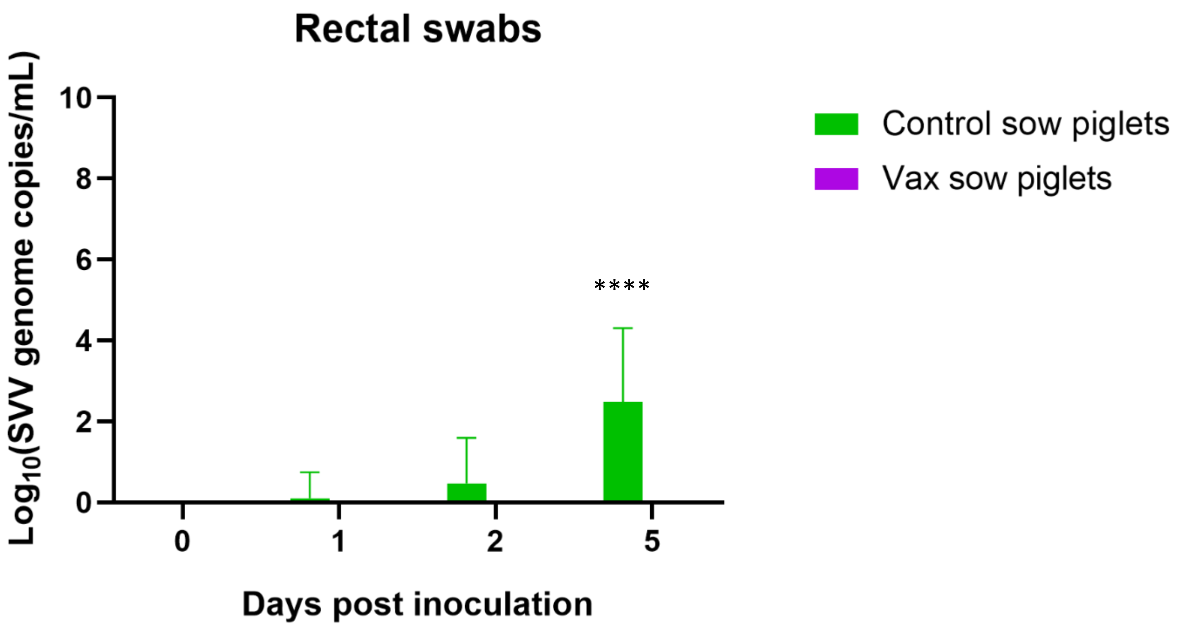

A
Total clinical score
Total clinical score
Days post challenge
0
3
5
7
14
0
1
2
3
Challenge
Vax+challenge
B
Rectal swabs
Log10(SVV genome copies/mL)
Days post challenge
0
1
2
3
5
7
14
0
2
4
6
8
10
Challenge
Vax+challenge
C
Serum
Log10(SVV genome copies/mL)
Days post challenge
0
3
7
0
2
4
6
8
10
Challenge
Vax+challenge

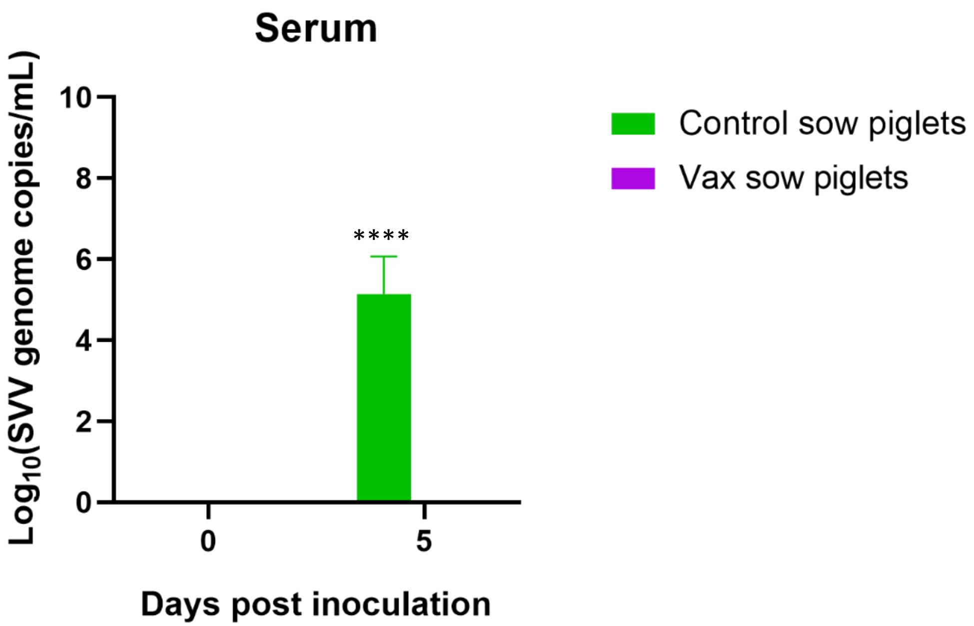
Figure 4

A



Rectal swabs
Log10(SVV genome copies/mL)
10
8
6
4
2
0
****
0
1
2
5
Days post inoculation
Control sow piglets
Vax sow piglets

B



Serum
Log10(SVV genome copies/mL)
10
8
6
4
2
0
****
0
5
Days post inoculation
Control sow piglets
Vax sow piglets