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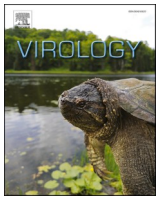

Evidence of vertical transmission of Senecavirus A in naturally infected sows

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ABSTRACT

Senecavirus A (SVA) is a Picornaviridae RNA virus that causes vesicular disease (VD) and transitory neonatal losses in pigs. The major ways SVA is spread are by oral, nasal, and feces. Vertical transmission of SVA was investigated during a VD epidemic in a farrow-to-finish herd in Brazil. Vesicular lesions were observed on sows before farrowing and on piglets within 24 h of birth. Analyses included RT-qPCR, viral isolation, sequencing, and virus-neutralization assays on serum, vesicular fluid, colostrum, and milk. Five out of ten sows were viremic before farrowing, and 46.7% of tested piglets had high viral loads in the first 24 h after birth. Infectious virus was detected in colostrum and milk from one postnatal sow. Despite high levels of neutralizing antibodies (nAbs) in piglet serum, colostrum, and milk, piglets were not protected from viremia and clinical illness. These findings support the vertical and congenital transmission of SVA.

1. Introduction

Senecavirus A (SVA) is a non-enveloped single-stranded, positive sense RNA virus, belonging to the genus *Senecavirus* within the *Picornaviridae* family (Hales et al., 2008). SVA causes an acute vesicular disease (VD) in pigs that is clinically indistinguishable from other VDs, such as foot-and-mouth disease (FMD), vesicular stomatitis virus (VSV) and vesicular exanthema of swine (VES) (Canning et al., 2016; Gimenez-Lirola et al., 2016; Montiel et al., 2016; Pasma et al., 2008; Vannucci et al., 2015). The virus was found retrospectively in samples collected since 1988 from pigs in the United States (U.S.), but the first detection of SVA associated with vesicular lesions was reported in 2007 in the U.S., from pigs imported from Canada (Pasma et al., 2008), and in 2010 from a boar from Indiana state (Singh et al., 2012). In November 2014, SVA-VD outbreaks were reported in Brazil, and in the U.S. in 2015. In these outbreaks, an increase in neonatal mortality was observed (Canning et al., 2016; Joshi et al., 2016b; Leme et al., 2015, 2016b; Vannucci et al., 2015). Following the first reports, several pig producing countries, like Canada, Colombia, China and Thailand, also reported SVA-VD outbreaks in their herds (Segales et al., 2017). A comparison between contemporary and historical SVA strains revealed a great genetic diversity, which could have contributed to the increase in the number of cases of SVA-VD (Joshi et al., 2016b, 2020). Based on molecular investigations, SVA was detected in pigs (showing vesicular lesions) from farms in multiple states in Brazil (Joshi et al., 2016b, 2020; Leme et al., 2015, 2016b; Saporiti et al., 2017), however, a retrospective serological study revealed that the SVA was not circulating in Brazil prior to 2014 (Saporiti et al., 2017).

The most common clinical findings in pigs are vesicles on the snout, in the oral mucosa and feet (dewclaw, coronary band and/or sole), associated with lameness, and lethargy (Canning et al., 2016; Segales et al., 2017). The vesicles rupture very quickly, evolving into ulcerated lesions on the skin that eventually heal in approximately 15 days (Leme et al., 2015; Montiel et al., 2016; Segales et al., 2017). SVA has a short-term viremia in serum for 1–10 days, which occurs along with the production of neutralizing antibodies (Maggioli et al., 2018). Tonsil is the primary site for SVA replication and persistence in the host (Maggioli et al., 2019). Stressor factors such as transportation, immunosuppression and parturition can lead to intermittent viremia, and virus shedding for up to two months (Maggioli et al., 2018, 2019).

Neonatal mortality, known as epidemic transient neonatal losses (ETNL), was also observed during SVA outbreaks and was mainly related

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to starvation and diarrhea (Canning et al., 2016; Leme et al., 2016a, 2016b). Several studies reported SVA infection in neonatal pigs, as early as one to two days after birth (Canning et al., 2016; Gimenez-Lirola et al., 2016; Leme et al., 2016a, 2016b). In one study, during an outbreak of VD in a sow farm in the U.S., SVA was detected in serum, tonsil and rectal swabs of piglets 12–24 h after birth (Tousignant et al., 2017), which suggests the vertical transmission of SVA.

The vertical transmission can occur by different routes and be classified as prenatal (transovarial, transplacental route and birth canal) and postnatal (colostrum/milk, oral/nasal secretions, feces, vesicular fluid from skin lesions) transmission. Congenital infections refer to those transmitted from mother to offspring during pregnancy (transplacentally) or during farrowing (peripartum). A transplacental congenital viral infection occurs when a virus crosses the placenta during pregnancy, potentially at any stage, leading to the fetus being exposed to the virus in utero. Consequently, the piglet is born with the infection already present in its body (Boucoiran et al., 2020). Peripartum congenital infections are those acquired by the fetus or newborn close to the time of birth. These infections can occur just before, during, or immediately after delivery. Previous studies considered that SVA transmission from sow-to-piglet occurs through the horizontal route (Maggioli et al., 2019), and the possibility of infection of suckling piglets during the prenatal or postnatal period was not demonstrated until recently (Kim et al., 2024). Leme et al. (2016b) described SVA infection in one-to five-day-old piglets characterized by the presence of oral vesicles and cutaneous lesions, disseminated immunolabeling of viral antigens, and detection of SVA RNA by RT-PCR. According to Maggioli et al. (2019), transmission of SVA from sow-to-piglet was associated with stressors, such as parturition, leading to intermittent viremia and virus shedding in infected piglets. The humoral immune response for SVA plays a major role in protecting pigs from the disease, consequently, neutralizing antibodies (nAbs) can reduce viremia, limit the spread of the virus to the tissues, delay and/or decrease the severity of the disease and also prevent reinfection (Joshi et al., 2016a; Whitton et al., 2005). Only recently SVA was described as a significant swine pathogen, and studies focused on the immune response after SVA infection and/or vaccination are extremely important (Fernandes et al., 2018; Maggioli et al., 2018).

In this study, we assessed the SVA RNA and the dynamics of antibody response in swine during an outbreak of VD in a farrow-to-finish farm. In addition, since the newborn piglets were in the viremic phase of SVA infection, we evaluated if the SVA infection could occur prenatally or if the colostrum and milk could serve as portal sites for oral postnatal vertical transmission.

2. Material and methods

2.1. Ethics approval

All experiments and methods were performed in accordance with the relevant guidelines and regulations. The experimental protocols used in this study were approved by the Ethics Committee on Animal Use (CEUA) from the Embrapa Swine and Poultry National Research Center (Protocol Number 001/2016). The study was carried out in compliance with the ARRIVE guidelines.

2.2. Clinical history and sample collection

During November 23 to December 9, 2020, a diagnostic investigation on a suspect case of vesicular disease was conducted on a farrow-to-finish research farm that belonged to the Brazilian Agricultural Research Corporation (Embrapa Swine and Poultry) and was located in a high-density swine production area in Santa Catarina, Brazil. The herd was composed of 150 sows, and the farrowing barn has one gestation room and five farrowing rooms. Seven days before the expected farrowing, 26 sows of mixed parities (1–6) were transferred from gestation to the farrowing room. This room was recently renovated, and it was the first time that sows were housed in the crates. One day before the expected farrowing (D-1), prostaglandin F2 alpha was administered in all sows and vesicular lesions were first observed in one of them. Twelve hours later, two other sows presented vesicles on the snout and coronary bands. On the expected parturition day, four more sows were affected. At the farrowing time, all 26 sows showed clinical signs of lameness and vesicular lesions. After birth, piglets were monitored daily for clinical signs and lesions. All sows were examined (feed intake, rectal temperature, vulval discharge and milk production) to exclude the possibility of puerperal disorders, mainly mastitis-metritis-agalactia syndrome. The official veterinary service was notified and SVA infection was confirmed by RT-qPCR in vesicular fluid and associated epithelium. Samples were negative to FMD, VSV and VES.

To follow the dynamic of SVA antibody response and viral shedding in colostrum and milk, 10 out of 26 infected sows that farrowed first were selected for monitoring and sample collection. Sera, vesicle fluid, colostrum and milk samples were taken from sows 152, 409, 575, 805, 924, 1127, 1220, 1362, 1474 and 1550. The day of parturition (just after the birth of the first piglet) was considered the day 0 (D0). Serum samples were collected from sows in the prepartum (D-1), D1, D3 and D7 postpartum. The three first-born piglets from each sow were selected, excluding piglets with low weight. Serum was collected on D1, D3, D7, D10 and D15. Colostrum was manually collected from all functional teats, just after the birth of the first piglet (D0), and before suckling. Milk samples were collected on D3, D5 and D7. In order to minimize the contamination of colostrum and milk, the sow teats were previously scrubbed with iodine alcohol, handled wearing disposable latex gloves and the samples were stored into sterile 50 mL conical tubes (Forner et al., 2021). Colostrum and milk samples were centrifuged at 1300×*g* for 20 min and the upper fat layer was discarded. Serum, colostrum and milk samples were stored at −80 °C for further analysis.

2.3. RNA extraction and RT-qPCR

Viral RNA was extracted from 50 μL of vesicular fluid, serum, colostrum, milk and from infected H1299 cells (SVA isolates) using the magnetic particle processor procedure (MagMAX™-96 Viral RNA Isolation kit, Applied Biosystems). RT-qPCR was performed using a commercial SVA RT-qPCR reagent targeting the conserved SVA 3D polymerase region (EZ-SVA, Tetracore Inc.). The cut-off value for SVA RT-qPCR was set on Cq 38.

2.4. Virus isolation

For virus isolation, vesicular fluid, colostrum, and milk samples were inoculated in H1299 non-small lung carcinoma cell line (ATCC CRL-5803). H1299 cells were grown in RPMI 1640 medium (Gibco BRL), supplemented with 10% fetal bovine serum (Gibco BRL), penicillin (100IU/mL), streptomycin (100 μg/mL) and gentamicin (50 μg/mL). Before inoculation in 24-well plates, the samples were pre-treated with 1x penicillin, streptomycin and fungizone (Gibco Antibiotic-Antimycotic). After adsorption for 1 h into semi confluent (60–80%) monolayer, 1 mL of complete growth medium (RPMI 1640, 10% fetal bovine serum and antibiotic) was added to each well. The plates were maintained for 5 days at 37 °C in a 5% CO_2 incubator, and inspected daily for visualization of cytopathic effect (Joshi et al., 2016b). After three blind passages, microplates were frozen and thawed twice and the homogenate was centrifuged at 3000 rpm for 5 min for debris removal. SVA isolation was confirmed by RT-qPCR and DNA sequencing.

2.5. SVA genome sequencing

The complete SVA genome sequence of one SVA strain isolated in this study was determined by the Sanger method using nine sets of overlapping primers (F1 to F9) (Joshi et al., 2016b). The PCR products

were subjected to electrophoresis in 1% agarose gels and purified by using QIAquick Gel Extraction kit (Qiagen). Sequencing reactions of the purified products were done with PCR primers using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), and further purified with BigDye XTerminator Purification kit (Applied Biosystems). The nucleotide sequences were determined using an ABI3130xl Genetic Analyzer. SVA genome sequences were analyzed and assembled using the Phred/Phrap/Consed softwares (http://www.phrap.org). Nucleotide sequence of the polyprotein was compared with other SVA sequences available in GenBank.

2.6. Virus neutralization assay

Initially, serial two-fold dilutions of heat inactivated sera, colostrum, and milk (1:40 to 1:40,960) were incubated for 1 h at 37 °C with 200 $TCID_{50}$ of SVA strain (BRMSA 2598) isolated in this study. H1299 cells were added to each well in a 96-well plate. After 48 h of incubation at 37 °C with 5% CO_2, the cells were fixed with 3.7% formaldehyde in PBS (pH 7.2) for 20 min at room temperature (RT) and washed three times with PBS. Cells were then permeabilized with 0.2% Triton-X and washed three times with PBS. After incubation with primary antibody (*anti*-SVA rabbit polyclonal antibody - gently provided by Dr. Diego G. Diel) diluted 1:3000 for 1 h at 37 °C, cells were washed as above and were incubated with goat anti-rabbit IgG (H + L) highly cross adsorbed secondary antibody - Alexa 594 conjugated (Invitrogen) diluted 1:1200 for 1 h at 37 °C (Joshi et al., 2016a). Finally, the nucleus of the cells was stained with DAPI (diamidino-2-phenylindole) (ThermoFisher) diluted 1:1000 for 10 min at 37 °C. Plates were read under a fluorescence microscope (EVOS M7000–10x magnification) and antibody titers were expressed as the reciprocal of the highest dilution of serum capable of completely inhibiting SVA replication. Negative and positive control sera were included in the assays.

2.7. Immunoglobulins quantification in colostrum and milk

All reagents were purchased from Bethyl Laboratories (Montgomery, TX, USA). The ELISA assays were performed to quantify the concentrations of the immunoglobulins IgG, IgM and IgA in colostrum as previously described (Forner et al., 2021).

3. Results

3.1. Clinical manifestations

Vesicular lesions were first observed in two of ten sows (ID 409 and 1220) 12 h before farrowing (D-1). Skin lesions were also verified in the remaining sows (ID 152, 575, 805, 924, 1127, 1362, 1474 and 1550) at the parturition day (D0). The main lesions observed in the affected sows were intact or ruptured vesicles on the snout and in the oral mucosa and also on feet (dewclaw, interdigital space, coronary band and/or sole) (Fig. 1). The vesicular lesions evolved to ulcerations with secondary bacterial infection. Other clinical signs which could be associated with SVA infection were also observed, such as lethargy, lameness, and mild diarrhea. All affected sows had normal colostrum and milk production. Abnormal vaginal discharge, or mastitis were not observed. Physiological hyperthermia associated with farrowing was observed (38.0–39.5 °C), with the temperature returning to basal levels in 24 h. The ten sows had 124 piglets born alive, and the mortality rate observed in the first 15 days was 16.1%. Not all piglets had clinical signs. In 27.4% (34/124) of the piglets, vesicles distributed around the coronary band of hooves were observed in less than 24 h up to 36 h after birth. In 3- to 5-day-old piglets, vesicles were observed on the snout (28.2%; 35/124) and ulcerative lesions on the coronary band, foot pads and hooves (46.7%; 58/124). Piglets also had mild diarrhea (46.8%; 58/124) with poor body condition, ill-thrift, anorexia, and lethargy (Fig. 2).

Fig. 1. Sows naturally infected with SVA. A–D: Ulcerative lesions on feet (dewclaw, coronary band and sole), E: Ruptured vesicle in the oral mucosa, F: Ruptured vesicle lesion on the snout.

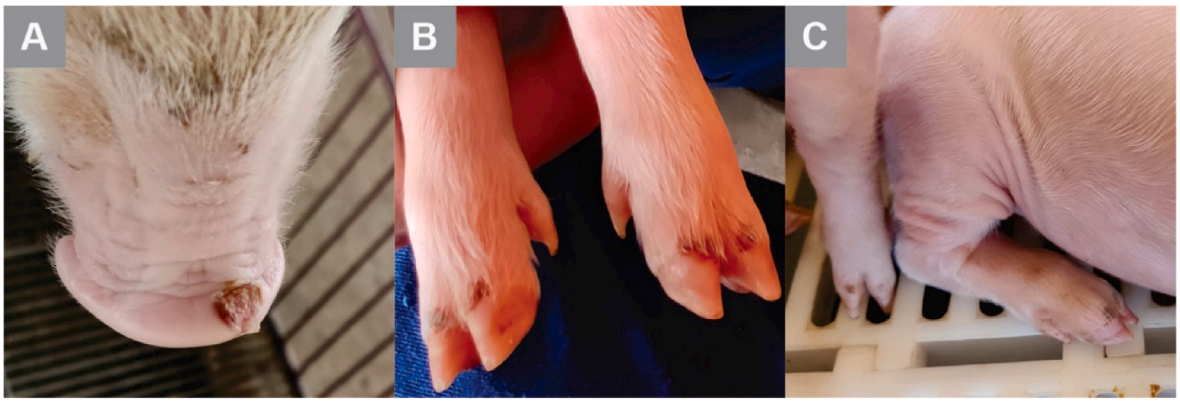

Fig. 2. Piglets naturally infected by SVA. A–C: Piglets with ulcerated vesicular lesions on the snout, and on feet.

3.2. Detection of SVA in pig samples and virus isolation

Vesicular fluids collected from three sows were positive to SVA by RT-qPCR with a cycle of quantification (Cq) varying from 12.64 to 15.93. The RT-qPCR results for SVA detection in serum, colostrum, and milk samples from sows and in serum collected from piglets are shown in Table 1. Before farrowing (D-1) five sows (ID 152, 805, 1127, 1474 and 1550) were SVA positive in serum (Cq 33.39–37.43), and two sows (ID 805 and 1474) remained positive until D3 (Cq 37.08–37.96). All sows were negative at D7. SVA was detected in the colostrum from three sows (ID 805, 1220 and 1474; Cq 35.48–37.69) and in milk from three sows (ID 924, 1220 and 1474; Cq 32.99–37.35). Interestingly, sows IDs 924 and 1220 had SVA positive colostrum and/or milk but the serum samples resulted negative. SVA excretion in milk was detected up to D3 (ID 1220), D5 (ID 924) and D7 (ID 1474) post farrowing. SVA was not detected either in serum or in colostrum/milk from sows 409, 575 and 1362.

All SVA viremic sows had viremic piglets. SVA RNA was detected in 46.66% (14/30) of piglet sera (from six litters) less than 24 h after birth (D1). Cq values ranged from 21.03 up to 37.96. Ten percent (3/30) of the piglet sera were positive until D7 (Cq 35.96–37.36).

SVA was isolated from a vesicular fluid sample after one passage in H1299 cells (Cq 14.97). The attempts for SVA isolation from colostrum and milk were performed on samples collected from four sows (ID 805, 924, 1220 and 1474) that tested positive by RT-qPCR. The virus was isolated in two out of eight samples after three passages in H1299 cells. SVA was isolated from the colostrum (Cq 33.27) and milk (Cq 32.39) from sow ID 1220 on D3 of lactation.

3.3. Genome sequencing

The virus identity was confirmed by genome sequencing. The

Table 1
Detection of SVA by RT-qPCR in serum samples from sows (D-1, D1, D3 and D7) and piglets (D1, D3, D7, D10 and D15), in colostrum (D0) and milk (D3, D5 and D7).

Sow ID	Sow serum				Colostrum	Milk			Piglets serum (3 per sow)				
	D-1	D1	D3	D7	D0	D3	D5	D7	D1	D3	D7	D10	D15
152	34.06	ND	ND	ND	ND	ND	ND	ND	35.79	ND	ND	ND	ND
									32.98	36.61	35.96	ND	ND
									ND	ND	ND	ND	ND
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
575	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
805	33.39	36.98	37.96	ND	37.69	ND	ND	ND	35.39	ND	ND	ND	ND
									35.47	36.07	ND	ND	ND
									ND	ND	ND	ND	ND
924	ND	ND	ND	ND	ND	32.99	35.99	ND	ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
1127	37.43	ND	ND	ND	ND	ND	ND	ND	21.03	30.56	ND	ND	ND
									23.04	33.19	ND	ND	ND
									22.52	31.36	ND	ND	ND
1220	ND	ND	ND	ND	36.41	37.21	ND	ND	36.97	ND	ND	ND	ND
									36.93	ND	ND	ND	ND
									37.96	ND	ND	ND	ND
1362	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND
1474	36.93	ND	37.08	ND	35.48	ND	36.61	37.35	23.92	35.75	37.36	ND	ND
									28.99	ND	ND	ND	ND
									25.03	32.93	36.03	ND	ND
1550	37.08	ND	ND	ND	ND	ND	ND	ND	37.01	ND	ND	ND	ND
									ND	ND	ND	ND	ND
									ND	ND	ND	ND	ND

ND: not detected. When the sample is positive, Cq value is shown.

complete genome sequence of SVA strain BRMSA 2598 (from vesicular fluid of sow) was deposited in GenBank under the accession number MZ456812. The SVA polyprotein was composed of 6546 nucleotides (nt), and the most similar (96.94%; 6346/6546 nt) SVA sequence was from a virus strain (MF615506) isolated in swine in Santa Catarina state in 2015. When comparing the SVA sequence from the current outbreak with SVA sequence (MF615507) isolated in the 2015 outbreak on the same farm, a similarity of 96.61% (6324/6546 nt) was found.

3.4. Virus neutralization assay

All ten sows presented nAbs against SVA with titers in serum ≧640 prior to farrowing (D-1). At seven days after farrowing, nAbs titers ranged from 1280 to 40,960. High levels of SVA nAbs were detected in the colostrum and milk from all sows. In most of them, nAbs levels in serum were associated with nAbs levels in the colostrum and milk (Fig. 3A–J).

Neutralizing antibodies were detected in serum from all piglets at D1 with titers ranging from 80 to 40,960. For six out of thirty piglets, a plateau curve of SVA nAbs was observed over time (Fig. 3P–S, T). The remaining 24 piglets showed a physiological decline in SVA nAbs over the first two weeks of life (Fig. 3K–O, Q-R). Antibody levels progressively decreased until undetectable levels (<40) in six piglets at 10-15 days-old. However, 24 piglets still had detectable SVA nAbs levels in serum at 15 days-old.

3.5. Quantification of immunoglobulins IgG, IgM and IgA in colostrum and milk

The average concentrations of IgG, IgM and IgA were higher in the colostrum than in milk. The IgG concentration in the colostrum was 74.41 mg/mL. Subsequently, the levels of IgG in milk decreased to 2.54 mg/mL on D3, followed by 1.59 mg/mL on D5, and to 1.55 mg/mL on D7. The IgA concentration in the colostrum was 28.22 mg/mL. The IgA levels in milk were 15.29 mg/mL on D3, 12.84 mg/mL on D5, and 17.79 mg/mL on D7. The IgM concentration in the colostrum samples was 30.97 mg/mL. The IgM concentration in milk was 9.66 mg/mL on D3, 9.58 mg/mL on D5 and 8.99 mg/mL on D7.

4. Discussion

SVA-induced vesicular disease has been reported in Brazilian pig farms since late 2014 (Leme et al., 2015; Vannucci et al., 2015). Different pig categories (weaned pigs, sows and finishing pigs) were affected and an increase in pig neonatal mortality, associated with SVA infection, was observed (Canning et al., 2016; Gimenez-Lirola et al., 2016; Leme et al., 2015, 2016a). SVA transmission occurs through contact with oral and nasal secretions, and feces from infected swine, as well as contact with fluid from recently ruptured vesicles (Joshi et al., 2016a), but vertical transmission has been poorly studied. Here, we investigated SVA shedding in the colostrum and milk from infected

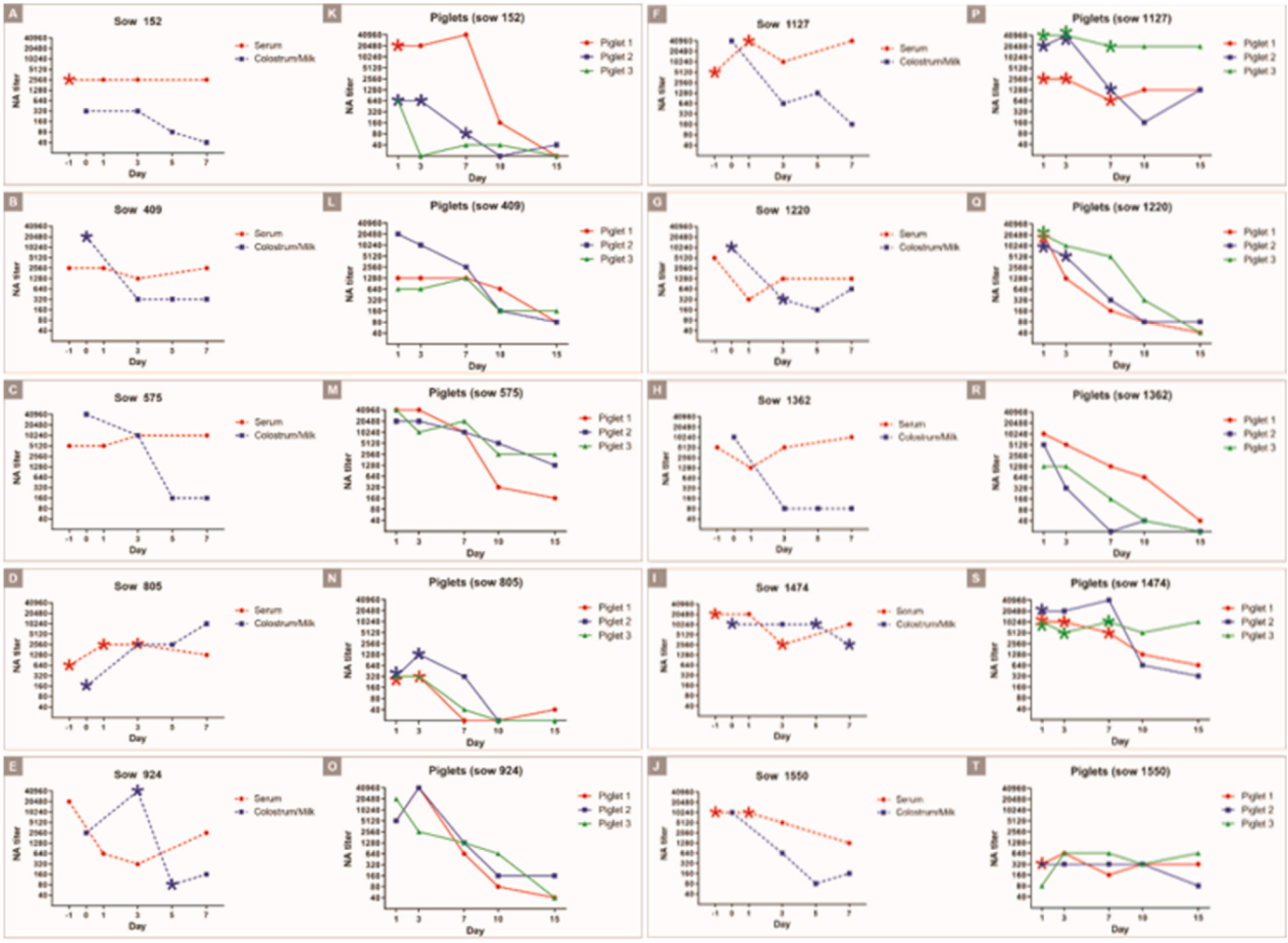

Fig. 3. Virus neutralization (VN) assay demonstrating neutralizing antibody responses against SVA in serum and colostrum/milk from sows and piglets. A-J are sows samples and K-T are piglets samples. Stars (*) pointed into the lines represent SVA detection by RT-qPCR on the referred moment.

sows, following an outbreak of vesicular disease in a farrow-to-finish farm. In addition, neutralizing antibodies against SVA were assessed in sows (serum and colostrum/milk) and in their littermates (serum). Initially, the sow's samples were evaluated by the official veterinary service to exclude infection by other viruses associated with vesicular disease. SVA infection was confirmed by RT-qPCR and sequencing in vesicular fluid and in the associated epithelium collected from infected sows, and samples were negative to FMD and VSV.

The studied farrow-to-finish farm experienced a previous outbreak of SVA-VD in November 2015. In that occasion, sows in the gestation and farrowing rooms presented clinical signs of VD and a mortality rate of 34% was registered for 3-to 5-day-old piglets, as well watery diarrhea and lethargy was also observed (Joshi et al., 2016a, 2016b). According to Vannucci et al. (2015), ETNL in Brazil have occurred in clusters, even in closed farms, and it was suggested by the authors that the development of herd immunity could prevent a clinical recurrence of SVA-VD. Here, we describe a five-year interval between two SVA outbreaks, therefore we can hypothesize that the herd was no longer immune to SVA. Additionally, the clinical presentation observed in piglets during the 2020 outbreak was different from that observed in 2015. In this recent outbreak, the mortality rate of neonatal piglets was 16.1%, and deaths occurred later, up to 15 days after birth. The pre-weaning mortality for this farm is approximately 18%. In addition, 27.4% of the piglets developed vesicular lesions within 24 h after birth, lasting for five days. Previous studies reported a 10–90% incidence of vesicular lesions in the field on affected farms (Baker et al., 2017; Leme et al., 2015; Tousignant et al., 2017). It is still unclear why not all pigs develop lesions after exposure to SVA. It has been suggested that lesion development may vary according to the virulence of the viral isolate (Fernandes et al., 2018; Zhang et al., 2020; Kim et al., 2024). Also, the minimum infectious dose of SVA to infected neonates (24–72 h after birth) is lower than to finishing pigs, which may affect the infection dynamics (Buckley and Lager, 2022). Other co-factors, such as viral or bacterial infections and an inappropriate environment, may alter the SVA pathogenesis (Kim et al., 2024).

Vertical transmission, mainly transplacentally, was described for other swine viruses, such as porcine parvovirus, porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, classical swine fever virus (Mims, 1981) and porcine epidemic diarrhea virus, this latter not associated with reproductive disorders (Ryu et al., 2022). Vertical transmission of SVA through the transplacental route, birth canal or colostrum/milk or other sow secretions was previously suggested but not confirmed. This possible route of transmission of SVA gained prominence when reports of increased piglet mortality emerged, as well cases of piglets showing viremia in the first days of life (Leme et al., 2016b; Maggioli et al., 2019). Recently, an experimental study provided support for the SVA transmission from sows to their offspring by the transplacental route, however, no clinical disease was noted in neonates or in most of the infected sows (Kim et al., 2024).

In our study, five sows that were viremic (SVA positive by RT-qPCR) at 12 h before parturition, had piglets that were viremic within 24 h after birth. The clinical findings in neonatal piglets were similar to those reported in the literature, and were characterized by an increase in the mortality rate, and detection of SVA RNA (Canning et al., 2016; Gimenez-Lirola et al., 2016; Leme et al., 2016a, 2016b). Unfortunately, it was not possible to sample the newborn piglets immediately after birth and prior to colostrum intake, which would have allowed us to confirm in utero infection by SVA. Nonetheless, detection of viremia and vesicular lesions in newborn piglets (within 24 h after birth) was a surprising finding, since previous studies conducted in experimentally infected pigs reported vesicular lesions on 4 days post infection (dpi) and short-term viremia between days 3 and 10 dpi (Fernandes et al., 2018; Joshi et al., 2016a; Maggioli et al., 2018; Montiel et al., 2016). Based on that, and if the infection of piglets occurred in the birth canal or shortly after birth, viremia was expected to occur in neonatal piglets on the third day of age. However, our findings showed that the highest viral loads were detected in piglets less than 24 h after birth (Table 1), reinforcing the possibility of congenital viral infection. The precise mechanisms of transplacental viral transmission, including the potential involvement of amniotic fluid and the placenta, require further investigation. In swine, due to epitheliochorial placentation, maternal antibodies are unable to cross the placenta, so newborn piglets are agammaglobulinemic at birth (Maciag et al., 2022). However, individual fetuses infected with SVA in utero may produce their own antibodies.

Additionally, we also suggest that different sow-to-piglet transmission routes may coexist, including postnatal transmission of SVA through colostrum/milk ingestion, since SVA was isolated from both colostrum and milk from infected sows. We demonstrated that the virolactia rate remained for 1 week (although with high Cq in RT-qPCR), and it declined 5 days after the onset of disease, coinciding with the increase in the level of nAbs in sows' serum. Herein, to avoid contamination, the colostrum samples were collected immediately after the birth of the first piglet and before the colostrum intake. Nevertheless, the risk of colostrum and milk contamination cannot be ruled out, since persistence of SVA in the environment of infected farms (due to shedding of SVA in oral and nasal secretions, and feces) for long periods has been reported (Joshi et al., 2016a). In regard to colostrum/milk quality, the IgG and IgA levels were similar to those described previously (Forner et al., 2021; Quesnel, 2011; Wagstrom et al., 2000). Conversely, high levels of IgM were detected in colostrum from infected sows, in agreement with other studies showing that an early IgM response against SVA is correlated with nAbs response (Maggioli et al., 2018). Interestingly, sows 575 and 1362 and their piglets were negative for SVA in all specimens collected by RT-qPCR and virus isolation, but they had nAbs for SVA. It is likely that the viremia had already ceased in the two sows, so no virus excretion was detected in colostrum/milk. According to Maggioli et al. (2018), high levels of virus-neutralizing (VN) antibodies can be detected in SVA-infected pigs as early as 5 days post-infection (p.i) and VN antibody titers remain elevated for a long period. In the case of sows ID152, 805 and 1550, SVA RNA was detected in serum and/or in colostrum/milk, although not all of their piglets tested positive. This may suggest that the sows were nearing the end of the viremia phase, or that piglet infection occurred during passage through the birth canal or postnatally. According to Kim et al. (2024), a variable viral detection profile was observed in piglets, with some non-viremic piglets testing positive for viral RNA in oral or rectal swabs, although such samples were not assessed in our study. Besides, two sows (IDs 924 and 1220) had detectable SVA in milk but not in serum. This variation in virus detection may be attributed to differences in the sensitivity of the detection methods that can vary between different types of biological samples, or to the timing of sample collection, which can influence the likelihood of virus detection. If the virus is present in the bloodstream only transiently but persists longer in milk, it could be detected in milk samples collected at a later time point. The transient viremia has been previously documented in pigs infected with SVA (Maggioli et al., 2019). Additionally, the immune system can compartmentalize viral infections, meaning that while the virus may be effectively controlled or neutralized in the bloodstream, it may persist in other tissues, such as the mammary glands (Zhang and Tscharke, 2016). Consequently, the virus might be undetectable in serum but still present in milk or colostrum. It remains to be discovered if this applies to SVA, but during lactation, some viruses are naturally shed in milk or colostrum as part of the virus clearance process.

With regard to SVA infection via colostrum and/or milk, the gastrointestinal tract of suckling piglets is still developing, so the ingestion of viral antigens by colostrum intake is potentially noxious, allowing viral antigens to penetrate the mucous epithelial barrier before gut closure (Donovan et al., 1994), causing infection or inflammatory reactions which may result in systemic disease. However, it is still unknown if SVA is inactivated by endogenous or exogenous factors, such as acidic pH and digestive enzymes by gastrointestinal tract and combined with mucus to form a chemical barrier to infection (Lipkin, 1985).

Two distinct patterns of nAbs in piglets were observed. The first pattern showed a decline of SVA antibodies detected over the first two weeks after birth (Fig. 3K–O, Q-R), in piglets from seven litters, consistent with a passive immune response (Rooke and Bland, 2002). A second pattern of nAbs was observed in piglets from the other three litters, where the antibody levels remained stable, suggesting that an active immune response had been initiated (Fig. 3P–S, T). Maternally derived antibodies from vaccinated sows are known to protect piglets from clinical disease (Yang et al., 2021). Even though nAbs levels in serum from piglets were high, viremia was detected in 46.7% of tested piglets within 24 h after birth along with the development of vesicular lesions. Our results indicate that high levels of nAbs were found in the colostrum and milk of acutely SVA-infected lactating sows and they were transferred to the piglets, however they did not protect them from viremia and clinical disease. As a result, the antibody titers they produce independently are very low. This low efficiency of nAbs in piglets may indicate infection of piglets before birth or, in the case of piglets with early acquired immune response, may indicate the presence of non-neutralizing antibodies or immature antibodies (Fernandes et al., 2018; Maggioli et al., 2018).

5. Conclusion

Our findings suggest the occurrence of the vertical transmission of SVA although we could not demonstrate that SVA RNA in sera from newborn piglets was present before the colostrum intake. The sow-to-piglet congenital transmission of SVA, either through the placenta or birth canal, is evidenced by the detection of viremia and the development of vesicular lesions in piglets within a few hours after birth. However, the precise mechanism of viral spread via amniotic fluid or the placenta requires further investigation. Colostrum and milk may serve as potential routes for SVA transmission to piglets as viable SVA has been successfully detected from these sources. Furthermore, the identification of multiple litters with viremic piglets on the first day of life, combined with the detection of SVA in colostrum samples, support the occurrence of intrauterine transmission. Our data provide important information about the transmission route of SVA and will be helpful in establishing a vaccine strategy for SVA.

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Data availability statement

The data that support this study are available from the corresponding author upon reasonable request. Source data are provided with this paper. The genome sequence is available in the GenBank repository (INSDC member repository), under the accession number MZ456812.

CRediT authorship contribution statement

Rejane Schaefer: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Danielle Gava:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Gabrielly E. Bombassaro:** Methodology, Investigation, Data curation. **Vanessa Haach:** Methodology, Investigation, Formal analysis, Data curation. **Shaiana S. Maciag:** Methodology, Conceptualization. **Ana Paula Bastos:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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