RESEARCHREPORT

SWINE HEALTH

pork checkoff

Title: Senecavirus A in sows: Impact of transportation stress on early development of vesicular lesions, transmission and recurrence of clinical disease in persistently-infected animals – **NPB #17-215**

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Industry Summary

Vesicular disease caused by Senecavirus A (SVA) has been an emerging concern on the swine industry due to its indistinguishable clinical presentation from high-consequence foreign animal diseases, including foot-and-mouth disease. The most common scenarios observed in SVA cases is the presence of vesicular lesions in finishing pigs and sows at or after arrival at packing plants from site with no previous history of the disease. The goal of the present research project was to understand the impact of transportation stress on early development of vesicular lesions, transmission and recurrence of clinical disease in persistently-infected sows.

The timeline for viremia and development of vesicular lesions were similar in stressed and non-stressed gilts. Virus transmission occurred to naïve-contact animals from infected animals 7 post-infection. These results were characterized by viremia (5 post-contact) and seroconversion (9 post-contact). Despite the lack of transmission observed after comingling infected-seeders and naïve animals at 21 and 35 post-infection of the seeders, gilts that have been recovered and cleared of the disease, resumed to shed the virus through feces and saliva 24 hours after stress (36 dpi). This result highlights the potential transmission of the virus under field conditions from asymptomatic animals that were previously assumed to be clear of the virus.

Key findings

- Stress did not induce earlier viremia or appearance of vesicular disease infected pigs
- Infected-seeder pigs transmitted the virus to naïve animals when comingled 7 days post-infection
- Asymptomatic-carrier gilts that have recovered and cleared of the infection resumed shedding SVA in feces and saliva 24 hours after stress
- Reoccurrence of vesicular disease was not observed in asymptomatic-carrier gilts after stress

Keywords: swine, Senecavirus A, sow, stress, pathogenesis, transmission

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Scientific Abstract

Senecavirus A (SVA) has been responsible for significant concern on the swine industry worldwide due to the similarities with other vesicular diseases, especially foot-and-mouth disease. The clinical identification of vesicular lesions in pigs triggers a foreign animal disease investigation, demanding time and resources for diagnostics and causing logistical problems for hog farmers and packing plants. Vesicular lesions are commonly detected in finishing pigs and sows after arrival at packing plants while often going undetected in the farms or buying stations. A hypothesis to explain these events and new outbreaks in sow farms is the potential persistence of the virus in the tonsil of infected animals after recovering from the clinical disease, and a possible effect of transportation stress on transmission and reoccurrence of lesions. The goal of the present study was to evaluate the ability of SVA infected animals to transmit the virus in three different stages of disease progression through direct contact with naïve animals after transportation stress. In addition, reoccurrence of vesicular disease and shedding of SVA after transportation stress in asymptomatic carrier animals was assessed. Eighteen gilts were allocated in four groups: SVA stressed (n=4), naïve-contact day 7 (n=4), naïve-contact day 21 (n=4), naïve-contact day 35 (n=4) and control (n=2). SVA stressed animals were inoculated intranasally with $2.7x10^8$ $TCID^{50}$. Naïve-contact animals were comingled with infected seeders on 7, 21 and 35 days post-inoculation (dpi) and kept in the same pen for 12 days until necropsy. Simulation of transportation stress was done on SVA stressed group using an experimental model based on National Pork Board's guidelines regarding temperature, space and feed and water restriction, with a duration of 8 hours. To mimic weather conditions in summer, the temperature of the room was raised to induce a low level of heat stress. To assess the effect of transportation stress in all timepoints, the transport model was performed in a manner that the end of the 8 hours coincided with the arrival of the naïve-contact animals on days 7, 21 and 35. Monitoring of viremia, serological IgG response and viral shedding by oral swabs, fecal swabs and tonsil swabs at 2, 5, 7, 9, 12 dpi or contact with infected seeder pigs was performed by RT-qPCR and IFA, and monitoring for clinical signs was performed daily. Infected seeder pigs were monitored on 14, 21, 28, 35, 42 and 49 dpi. Transmission occurred to naïve-contact animals from day 7, with 4/4 animals showing viremia on day 5 post-contact. All naïve-contact comingled at day 7 had positive IFA results 9 days post-contact. All SVA-stressed animals were shedding 7 dpi, but only 1/4 seeder and 0/4 were shedding 21 and 35 days post-inoculation, respectively. Vesicles were detected in 2/4 of inoculated animals on days 4 and 5 post-inoculation, and reoccurrence of lesions did not happen after any mock transportation procedure. In conclusion, stress appeared not to be determinant on SVA transmission, and the decrease of fecal shedding in SVA-stressed animals over time coincides with the lack of transmission to naïve animals as they were put in contact on days 21 and 35.

Introduction

Vesicular disease caused by Senecavirus A (SVA) has been an emerging concern on the swine industry due to its indistinguishable clinical presentation from high-consequence foreign animal diseases, including *foot-and-mouth* disease. One of the most common scenarios observed in SVA cases is the presence of vesicular lesions in finishing pigs and sows at or after arrival at packing plants. These findings raise questions such as (i) whether the animals are being exposed during or after loading at the finishing sites, (ii) whether a stress event including transportation would induce a short incubation period and result on early development of vesicular lesions, and (iii) whether the detection of lesions in packing plants are the tail end of an infection in finishing pigs that went unobserved. The overall goal of this proposal is to determine the role of transportation stress on early development of vesicular lesions, transmission and recurrence of clinical disease in sows persistently infected with Senecavirus A.

Research objectives

Objective 1: Evaluate the primary sites for virus replication and time (in hours) for development of vesicular lesions in sows experimentally infected with SVA.

Objective 2: Compare the early distribution of virus replication and development of vesicular lesions in sows experimentally infected with SVA after transportation stress and under minimal stress conditions.

> *Research question*: Are there differences on primary sites for SVA replication and time required for development of vesicular lesions after transportation and under minimal stress conditions?

Objective 3: Evaluate the transmission of Senecavirus A in naïve sows in contact with three categories of infected animals after transportation stress:

(i) clinically-affected and shedders (7 days post-inoculation);
(ii) clinically-recovered and shedders (35 days post-inoculation);
(iii) clinically-recovered and non-shedders, but persistently-infected (50 days post-inoculation).

> *Research question*: Are persistently-infected sows (asymptomatic carrier) able to transmit SVA to naïve animals after transportation stress?

Objective 4: Assess the recurrence of clinical disease and shedding of Senecavirus A in persistently-infected sows after transportation stress.

> *Research question*: Does transportation stress induce reoccurrence of vesicular disease and shedding of SVA in persistently-infected sows (asymptomatic carrier)?

Materials & Methods

Thirty-four gilts were allocated in six groups: SVA stressed (n=12), SVA non-stressed (n=8), naïve-contact day 7 (n=4), naïve-contact day 21 (n=4), naïve-contact day 35 (n=4) and control (n=2). SVA stressed and non-stressed animals were inoculated intranasally with 2.7×10^8 $TCID^{50}$ at hour 0, and at the same time simulation of transportation stress was started on SVA stressed group using an experimental model based on National Pork Board's (NPB) guidelines regarding temperature, space and feed and water restriction, with a duration of 8 hours. During the 8 hours of mock transportation the temperature of the room was raised according to its relative humidity at the time to induce a low level of heat stress, while mimicking temperature conditions in the summer. Guidelines for temperature range were followed using NPB's Livestock Hot Weather Safety Index, available in the Transport Quality Assurance Version 6 Handbook, so that conditions would fall into the "alert zone". Two animals from each of the inoculated groups were euthanized at 6, 12, 24 and 48 hours post inoculation (hpi) to evaluate the effect of minimal stress conditions on the early pathogenesis of SVA. Four animals from SVA stressed group were kept to be used as infected seeders for the remaining time of the study. Naïve-contact animals were comingled with infected seeders on 7, 21 and 35 days post-inoculation (dpi) and kept in the same pen for 12 days until necropsy. To assess the effect of transportation stress in all timepoints, the transport model was performed repeatedly in infected seeders in a manner that the end of the 8 hours coincided with the arrival of the naïve-contact animals on days 7, 21 and 35.

Early pathogenesis was evaluated by collecting tissues from four different tonsils (soft palate, pharyngeal, paraepiglottic and lingual tonsils) and testing by SVA RT-qPCR, and the distribution of replicating virus was assessed by *in situ* hybridization (ISH). Systemic distribution of SVA was assessed by PCR in regional lymph nodes (submandibular, retropharyngeal and tracheobronchial) and tissue homogenate (pool of lung, heart, spleen, kidney). Collection of samples in live animals during the first 48 hours of the study included fecal swabs and sera for PCR in animals euthanized at 6hpi; and oral swabs, fecal swabs, tonsil swabs and sera for PCR in remaining animals at 12, 24 and 48hpi, at the same time of euthanasia of two animals from each inoculated group.

Monitoring of viremia, serological IgG response and viral shedding was performed by oral swabs, fecal swabs and tonsil swabs at 2, 5, 7, 9, 12 dpi or contact with infected seeder pigs by RT-qPCR and an indirect immunofluorescence assay (IFA); and monitoring for clinical signs was performed daily. Infected seeder pigs were also monitored on 14, 21, 28, 35, 42 and 49 dpi. On days 21 and 35, infected seeder pigs had samples taken twice in each day, before and after mock transportation, to assess the effect of stress.

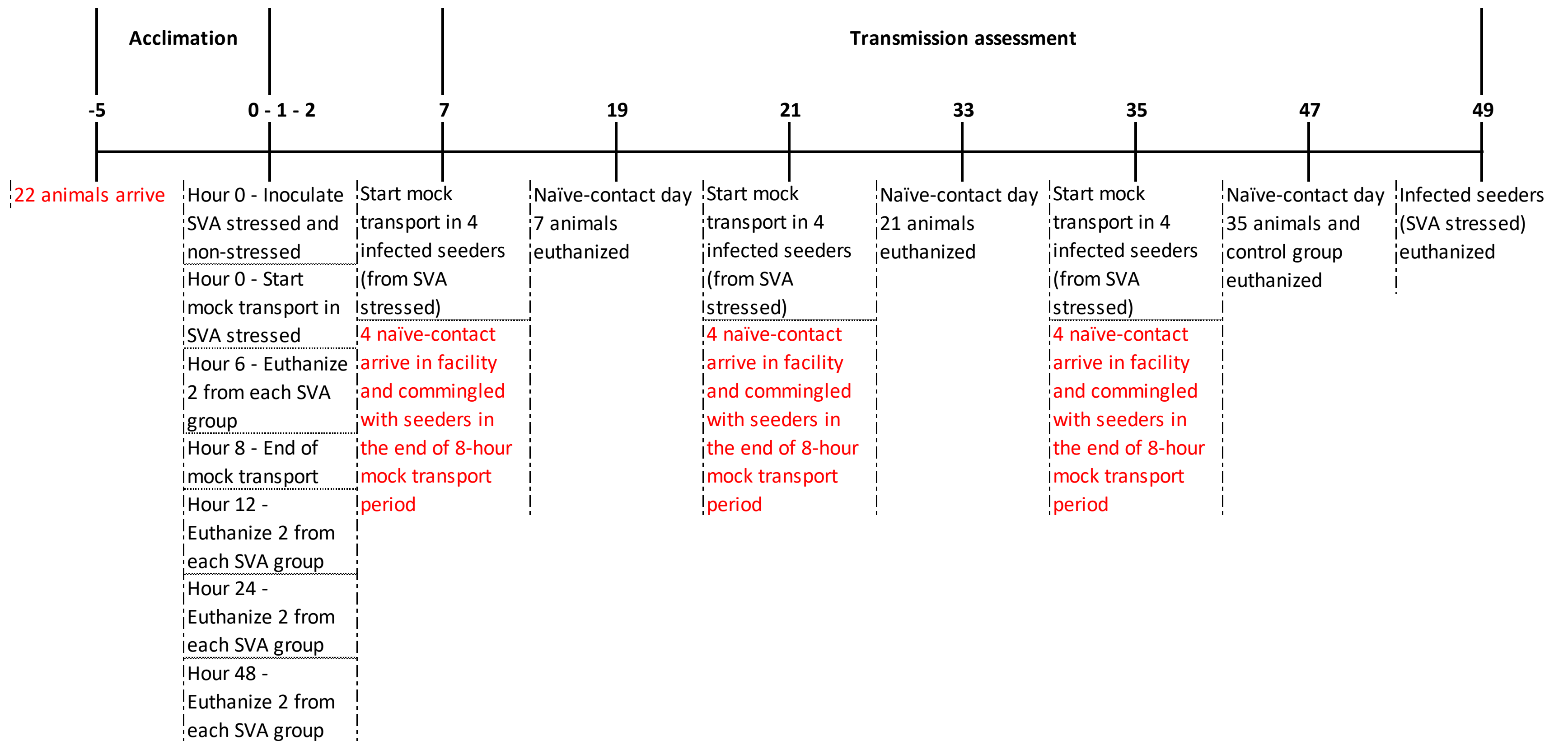

Figure 1 – Representation of the experimental design and timeline (days).

Results and Discussion

All the pigs were *Senecavirus A* specific antibodies negative through IFA at the beginning of the study or when brought in for contact with infected seeders; and were also PCR negative in oral swabs.

Objective 1 - Are there differences on primary sites for SVA replication and the time required for development of vesicular lesions after transportation and under minimal stress conditions?

All pigs euthanized at 6hpi had PCR negative fecal swabs and sera.

At 12hpi, only 1/6 SVA non-stressed animal had PCR positive oral (Ct 34) and fecal (Ct 36) swabs; 5/6 had positive tonsil swabs with a mean Ct value of 34; and all 6 animals had PCR negative sera. On SVA stressed group, 5/10 animals had PCR positive oral swabs, 4/10 had positive fecal swabs and 8/10 had positive tonsil swabs with average Ct values of 33, 34 and 34 respectively; and all animals had PCR negative sera.

At 24hpi, 4/4 SVA non-stressed had PCR positive oral swabs, 4/4 had positive tonsil swabs and 3/4 had PCR positive sera with average Ct values of 26, 26 and 31 respectively; and only 1/4 had a positive fecal swab with a Ct value of 29. On SVA stressed group, 8/8 had positive oral swabs, 3/8 had positive fecal swabs, 8/8 had positive tonsil swabs, and 6/8 had PCR positive sera with average Ct values of 28, 32, 26 and 31 respectively.

At 48hpi, 2/2 pigs on SVA non-stressed pigs had positive results for oral swabs, fecal swabs, tonsil swabs and sera with average Ct values of 26, 24, 28 and 30 respectively. On SVA stressed group, 5/6 had positive oral swabs and fecal swabs, 5/6 had positive fecal swabs, and 6/6 had positive tonsil swabs and sera with average Ct values of 27, 30, 27 and 29 respectively.

Table 1 shows PCR and ISH results in different collected tissues at 6, 12, 24 and 48 hours, regarding sites where viral RNA and viral replication was present in these different timepoints. Tonsils of the soft palate appear to be important sites for viral replication all times, showing high positivity rate even at 6hpi when Ct values were higher. Paraepiglottic tonsils and pharyngeal tonsils also showed positive PCR and ISH results at all times, with decreasing Ct values over time. Lingual tonsils also had decreasing Ct values over time, but viral replication was only firstly detected at 12hpi in SVA stressed group animals, and then in animals from both groups after 24hpi. Tracheobronchial lymph-nodes also had viral replication detected firstly by ISH on 2/2 SVA stressed animals at 24hpi, as in the coronary bands of 2/2 SVA stressed animals at 48hpi. Snout skin only showed positivity by ISH at 48hpi in 1/2 and 2/2 animals from SVA non-stressed and SVA stressed groups respectively.

Inoculated animals did not develop lesions during the first 48 hours of the study, when the early pathogenesis was evaluated. Two out of four of the animals from SVA stressed group that remained for the rest of the study developed lesions. On day 4, one animal had an erythematous lesion on the snout, which developed into a vesicle that was visible on day 5 and ruptured on day 7 post inoculation. On day 5, the other animal had an eroded vesicular lesion on the snout, which

was not noted in the previous days. No lesions were seen on the feet of the animals, and no animals showed signs of lameness or lethargy during the clinical phase.

Objective 2 - Are persistently-infected sows able to transmit SVA to naïve animals after transportation stress?

The four animals that remained from the SVA stressed group, from now on referred to as infected seeders, started showing PCR positive sera on day 1, with 2/4 animals yielding positive Ct values with an average of 32. From then on, 4/4 had positive sera on day 2, 3/4 had positive sera on days 5 and 7, with average Ct values of 31, 31 and 34 respectively. On day 9, 1/4 had a positive Ct value of 35. The first positive serological result from IFA appeared on day 7 in 1/4 pig with a titer of 1:80; and from day 9 forward all 4 animals showed positive IFA values with a titer of 1:80. PCR positive sera and positive IFA values indicate the success in the inoculation in these four animals.

Shedding of the virus was evaluated by the means of oral, fecal and tonsil swabs. The mean Ct values on oral swabs were 29, 27, 28, 25, 28 and 33 on days 1, 2, 5, 7, 9 and 14 respectively, with all samples being positive except one on day 2. Only one animal had a positive fecal swab on day 1 (Ct 30), and the mean Ct values for days 2, 5, 7, 9, 14 and 21 were 28, 31, 32, 31, 33 and 35 respectively, with only one negative sample on day 21. Tonsil swabs remained consistently positive until day 14, when the first animal showed a negative result, and only 1/4 pig had a PCR positive tonsil swab. Mean Ct values were 25, 28, 27, 27, 28 and 34 respectively on days 1, 2, 5, 7, 9 and 14, and the one PCR positive sample from day 21 had a Ct of 28.

Tonsil scraping was done on all four infected seeders after day 21 to attempt to detect the virus being harbored in the tonsils of the soft palate. This sampling method enabled the detection of 3/4 animals with PCR positive results and an average Ct value of 32 at the time of commingling with naïve-contact animals from day 21, while only 1/4 animals were positive by tonsil swabs and fecal swabs, and no animals had positive oral swabs. Consecutive tonsil scrapings yielded positive results in 3/4 animals on day 28 (mean Ct value of 32) and 1/4 animal on day 35 and 48 with average Ct values of 31 on both days.

At necropsy, infected seeder pigs were still positive in many tissues, when 4/4 animals had PCR positive tonsils of the soft palate, pharyngeal tonsils, lingual tonsils, retropharyngeal lymph nodes and submandibular lymph nodes (average Ct values of 29, 31, 32, 24 and 28 respectively); and 3/4 showed positive results in the epiglottic tonsils, with an average Ct of 30.

Transmission of SVA only occurred to animals in the naïve-contact group from day 7, when shedding of virus was high in infected seeders. Very similarly to the inoculated animals, these naïve-contact animals had 3/4 positive results from oral and tonsil swabs 2 days after exposure (average Ct values of 30 for both); 2/4 had PCR positive sera with an average Ct of 30, and all animals had negative fecal swabs. Five days after exposure, 4/4 naïve-contact animals from this group had PCR positive oral swabs, tonsil swabs and sera, with average Ct values of 30, 25 and 30 respectively; and 3/4 had positive fecal swabs with an average Ct of 32. Seven days after

exposure, all animals showed positive results in all samples, and 2/4 showed positive serological results through IFA, tittered at 1:80. On days 9 and 12 after exposure, 4/4 naïve-contact pigs had IFA positive results, tittered at 1:80. No clinical signs were seen on animals from naïve-contact 7 group. Naïve-contact animals from days 21 and 35 did not show positive PCR or IFA results.

Objective 3 - Does transportation stress induce reoccurrence of vesicular disease and shedding of SVA in persistently-infected sows (asymptomatic carrier)?

Vesicular lesions were only seen in two out of four animals from SVA stressed group that remained for the rest of the study (infected seeders). On day 4, one animal had an erythematous lesion on the snout, which developed into a vesicle that was visible on day 5 and ruptured on day 7 post inoculation. On day 5, the other animal had an eroded vesicular lesion on the snout, which was not noted in the previous days. No lesions were seen on the feet of the animals, and no animals showed signs of lameness or lethargy during the clinical phase.

After undergoing the mock transportation procedure on day 7, when the animals were considered as "clinically affected and shedders", no new lesions were developed. Shedding did not appear to be affected, as PCR results from days 7 and 9 showed Ct values of 25 and 28 in oral swabs and 32 and 31 in fecal swabs respectively.

After mock transportation on day 21, when animals were considered "clinically recovered and shedders", no lesions developed. This period of 21 days post inoculation is when the animals were expected to be reaching minimal shedding levels, as described by Maggioli et al. (2017). Animals were sampled immediately before and again immediately after mock transportation, and all PCR results from oral swabs were negative in both timepoints; 3/4 and 1/4 animals showed positive fecal swabs before and after mock transport respectively, with relatively high Ct values of 35 in average before the procedure and another Ct of 35 in the one positive sample from after the procedure. On day 28, all fecal swabs were negative; 1/4 animals had a Ct of 35 in an oral swab; 2/4 animals had an average Ct of 34 in tonsil swabs and 3/4 had and average Ct of 32 in tonsil scrapings.

After day 35, when the last mock transportation procedure was done and the animals were considered "clinically recovered and non-shedders, persistently-infected", again no lesions developed. At the sampling before the mock transportation, all four animals had negative oral, fecal and tonsil swabs, and 1/4 had a Ct of 31 in a tonsil scraping. After the procedure, the only positive sample was the tonsil scraping from the same animal that was positive before, now with a Ct of 32. Interestingly, this same animal with a positive tonsil scraping showed positive oral swabs, fecal swabs, tonsil swabs and tonsil scrapings on the next day (day 36), with Ct values of 35, 33, 33 and 32 respectively. This same animal showed a positive tonsil scraping on day 48, right before euthanasia, with a Ct of 31. All other samples from all animals on days 42 and 48 were negative.

Table 1: PCR results and ISH results regarding distribution of replicating viruses in different tissues at 6, 12, 24 and 48 hours post-inoculation.

Hours post-inoculation	6 hours post-inoculation				12 hours post-inoculation				24 hours post-inoculation				48 hours post-inoculation			
Animal ID	8313	8520	8354	8538	8349	8320	8376	8526	8282	8322	8235	8502	8124	8567	8271	8380
Group	Non-stressed	Non-stressed	Stressed	Stressed	Non-stressed	Non-stressed	Stressed	Stressed	Non-stressed	Non-stressed	Stressed	Stressed	Non-stressed	Non-stressed	Stressed	Stressed
Nasal turbinate (Ct Value)	27.8	33.92	34.49	27.46	30.35	32.21	26.51	30	28.67	27.86	30.6	33.37	27.1	25.65	27.14	27.15
Nasal turbinate	++	++	++	++	+++	++	+	+++	-	-	-	-	-	-	-	-
Tonsil of the soft palate (Ct Value)	30.02	31.49	34.01	32.87	32.22	26.69	28.13	26.31	22.2	17.87	23.09	22.32	18.1	22.69	26.7	20.36
Crypt (epithelium)	+++	++	++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	++	+++
Interfollicular	+++	+++	+++	+++	+++	++	++	+++	++	++	++	++	+++	+++	++	++
Lymphoid follicles	-	-	-	-	-	-	-	-	-	-	-	-	++	-	++	-
Paraepiglottic tonsil (Ct value)	25.14	31.7	30.51	28.33	27.47	22.43	19.63	27.42	25.29	23.62	26.05	25.16	17.49	19.89	17.2	14.93
Crypt (epithelium)	+	-	-	-	++	++	+++	++	++	+++	++	++	++	+++	+++	++
Interfollicular	++	++	-	-	++	++	+++	+++	++	++	++	+++	+++	+++	+++	-
Lymphoid follicles	++	++	++	+	+	-	+	-	-	+	++	++	++	-	-	-
Pharyngeal tonsil (Ct Value)	27.8	26.27	33	25.4	25.47	25.43	26.84	23.61	20.38	21.18	21.02	26.8	21.39	20.88	21.19	23.35
Interfollicular	+++	++	+++	+++	++	+++	++	++	+++	+++	+++	+++	+++	++	+++	++
Lymphoid follicles	-	++	++	+	+	-	-	-	+	+	-	-	++	++	-	-
Lingual tonsil (Ct Value)	33.56	32.45	33.25	31.42	29.76	31.8	26.19	28.46	22.42	25.82	20.79	28.14	17.26	22.53	23.24	20.23
Interfollicular	-	-	-	-	-	-	++	++	++	++	++	++	++	++	++	++
Lymphoid follicles	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++
Retropharyngeal LN (Ct Value)	37.08	34.49	Neg	38.04	31.85	31.78	31.4	31.01	19.71	28.5	21.26	31.07	24.49	26.22	20.93	17.38
Retropharyngeal LN	-	-	-	-	++	++	++	++	+++	++	+++	++	+++	+++	+++	+++
Submandibular LN (Ct Value)	33.33	33.92	Neg	33.59	31.49	33.78	31.4	30.1	23.76	29.14	28.66	24.43	20.3	21.18	19.09	21.98
Submandibular LN	++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
Tracheobronchial LN (Ct Value)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	32.36	Neg	33.2	35.61	26.58	32	25.88	23.02
Tracheobronchial LN	-	-	-	-	-	-	-	-	-	-	++	++	+++	+++	+++	+++
Snout (Ct Value)	37.23	35.07	Neg	35.8	Neg	Neg	34.23	32.47	Neg	32.09	33.46	32.87	31.15	28.67	28.95	28.47
Snout	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	++
Coronary band (Ct Value)	Neg	Neg	Neg	Neg	37.87	Neg	34.16	34.41	Neg	35.65	35.36	Neg	34.58	34.52	29.55	26.79
Coronary band	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++

Legend:
PCR results represented by numbers.
(-) negative
(+) focal distribution
(++) multifocal distribution
(+++) diffuse distribution

Conclusions

Based on the initial concern from the swine industry to explain the unexpected appearance of vesicular lesions in packing plants from farms with no history of SVA infection, we evaluated the ability of SVA infected animals to transmit the virus in three different stages of disease progression through direct contact with naïve animals after transportation stress. In addition, we also assessed the reoccurrence of vesicular disease and shedding of SVA after transportation stress in asymptomatic carrier animals. Transmission occurred to naïve-contact animals from day 7, with 4/4 animals showing viremia on day 5 post-contact. All naïve-contact comingled at day 7 had positive IFA results 9 days post-contact. Vesicles were detected in 2/4 of inoculated animals on days 4 and 5 post-inoculation, and reoccurrence of lesions did not occurred in any time points after stress procedures.

Despite the lack of transmission observed after comingling seeders and naïve animals at 21 and 35 post-infection, gilts that have been recovered and cleared of the disease resumed to shed the virus through feces and saliva 24 hours after stress (36 dpi). This result highlights the potential transmission of the virus under field conditions from asymptomatic animals that were previously assumed to be clear of the virus.