Title: Assessing Senecavirus A shedding and transmission in growing pig populations

Primary investigator
Guilherme Milanez Preis, DVM
Graduate Research Assistant – Ph.D. Candidate
University of Minnesota
College of Veterinary Medicine
385 An Sci/Vet Med
1988 Fitch Ave
St. Paul, MN 55108

Co-investigator
Cesar A Corzo, DVM, MSc, Ph.D.
Allen D Leman Chair, Swine Health, and Productivity
University of Minnesota
College of Veterinary Medicine

Statement of the problem

Senecavirus A (SVA) has been linked to several vesicular disease outbreaks in pigs worldwide and is responsible for a constant increase in the yearly number of foreign animal disease (FAD) investigations conducted by the United States Department of Agriculture (USDA). Very little information is currently known about this virus's epidemiology and transmission. Previous AASV foundation-funded research showed that SVA RNA could be found in processing fluids for an average of 11.8 weeks after an outbreak is detected in a sow farm. Moreover, heat-check boars were shown to be potential SVA carriers that may infect incoming naïve gilts over time.

Reports from swine practitioners state that many SVA cases do not have an obvious source. Up to this date, there is no published information on SVA shedding and transmission in growing-pig populations weaned from recently broken, SVA-positive sow farms. Due to the nature of this disease, SVA transmission events could be happening silently and without any apparent clinical signs in growing-pig farms. SVA shedding and transmission in growing pigs weaned from SVA-positive sow farms could be assessed using molecular diagnostics by testing serum and oral fluids.

Objectives

- Describe SVA shedding patterns over time in growing-pig populations
- Assess the state of infection in piglets after comingling into the nursery phase

Data collected and laboratory testing

A 2,800-sow farm broke with SVA on October 29, 2021. It is unknown whether this farm had any previous SVA exposure before this event. This farm was selected for this longitudinal observational study, and five different groups of weaned piglets were

followed during their grow-finish phase. In order to assess viral shedding and viremia, eight consecutive oral fluids (OF) and one blood sampling collections were requested over time, starting when the weaned piglets arrived in the wean-to-finish barns (WF). Processing fluids (PF) samples were requested from the sow farm for each enrolled group of piglets. Additionally, environmental samples (Swiffer) were requested from the wean-to-finish sites before the placement of the piglets. All wean-to-finish barns single-sourced the piglets from the enrolled sow farm, and each barn was filled in one day with a single group of weaned piglets.

The sampling scheme in the WF barns included the collection of four OF samples from each weaned group at the week of arrival; 1 week post-placement of piglets in the WF barn; 3 weeks post-placement; 4 weeks post-placement; 8 weeks post-placement; 11 weeks post-placement; 15 weeks post-placement; and, lastly, 1 week before marketing the finished pigs. Each of the four ropes was hung in the middle of two pens to acquire oral fluids samples from animals in eight different pens, increasing the chances of detection. All groups of pigs were vaccinated for *Mycoplasma hyopneumoniae* 3 weeks post-placement; therefore, OF samples were collected at weeks 3 and 4 post-placement in an attempt to detect viral shedding due to vaccine-related stress and potential transmission caused by the movement of personnel between pens. Since most SVA-infected pigs develop viremia for approximately 10 days after infection[1,2], 60 blood samples were collected 1 week post-placement in an attempt to detect viremia caused by transmission events after comingling at the week of arrival in the WF barn. Details on each group of pigs can be seen in Table 1.

The following number of samples were tested by SVA rRT-PCR: 299 blood samples, 136 oral fluids (OF), 16 processing fluids (PF), and 12 environmental (Swiffer) samples. Blood samples were centrifuged, and serum was tested in pools of 2 samples. Swiffer and OF samples were tested individually. No serology testing was performed due to the potential confounding caused by maternal immunity transfer.

Test results are summarized in Figures 1 and 2. Unfortunately, it was not possible to collect all the intended samples, as seen in Figure 1.

Significant findings and recommendations

The PF results agree with earlier findings. SVA RNA was found in PF samples from groups 2 to 5, which could mean that piglets weaned up until ten weeks after outbreak detection were exposed to SVA infection. PF samples were not collected during the week of the SVA outbreak, so PF results are unavailable for Group 1. However, since all other groups had SVA-positive processing fluids, and based on the data from previous research, it would be reasonable to assume that Group 1 would have had positive processing fluids if it had been collected.

Environmental (Swiffer) samples from before the pigs were placed in the barn were only available from groups 2 and 5 (Figure 1). Groups 2 and 5 had negative results from the Swiffer samples. Group 1 had Swiffer samples collected after placing the pigs in the

barn, with PCR-positive results, indicating that animals were shedding the virus and contaminating the barn environment. It is unknown whether the barn environments from Groups 1, 3, and 4 were SVA-positive before the piglets arrived in the facility since the Swiffer samples were not collected.

SVA shedding in the growing phase was only detected by OF testing in group 1 (weaned 25 days after outbreak detection), suggesting that virus transmission may occur post-weaning. OF results from Group 1 were positive at the week of arrival, 1 week post-placement, and 3 weeks post-placement, with Ct values ranging from 30 to 35. OF testing results from weeks 4 and 8 post-placement had Ct values above 36, which can be classified as suspect results. However, the increasing Ct values above the 36 Ct threshold can be due to decreasing levels of shedding over time after most or all animals are exposed to the virus.

Interestingly, Groups 2, 3, 4, and 5 had only negative OF results throughout the entire period despite having positive results from the PF collected in the farrowing room. An increase in maternal immunity transfer could explain this as time passes after an outbreak on a sow farm. However, it is unknown whether the enrolled sow farm had any SVA immunity from previous exposures to SVA, and antibody levels were not assessed, so very little can be concluded from this perspective. The possibility of the weaned piglets being SVA-positive cannot be discarded since the positive PF results in the farrowing room are strong indicators of SVA exposure. Even if a small proportion of weaned piglets are positive, there is a possibility that stressful events later in the life of the pigs trigger shedding events[3] that could lead to SVA transmission and infection. Another study targeting the sampling and testing of sows and weaning piglets over time after the sow's SVA exposure needs to be performed to understand better the relationship between the time between an SVA outbreak and the SVA status of weaned pigs.

All serum PCR results were negative for the 60 piglets collected 1 week post-placement of all groups of pigs. It is unknown whether our negative findings in sera are due to maternal immunity or because most pigs were already infected during the suckling period, and viremia had ended at the time of sample collection. Alternatively, transmission could have happened at low levels in the second week of the growing phase, and our sample size was not large enough to detect viremic pigs.

Overall, this project confirmed that pigs can shed and transmit SVA in the grow-finish phase after exposure in the farrowing room.

How will these findings assist the practicing veterinarian?

This project has advanced the knowledge of the epidemiology of SVA in pigs after an outbreak on a sow farm. This study replicated the findings from a previous study, showcasing the usefulness of PF testing to assess SVA circulation in the farrowing room. Furthermore, these findings demonstrate that weaned piglets can shed SVA

constantly for up to 3 weeks after entering a wean-to-finish barn, but this period may be as high as 8 weeks.

This information can be used by a practicing veterinarian interested in assessing the SVA status of piglets over time after an outbreak on a sow farm. OF samples can be used to evaluate shedding in the grow-finish phase, but other sampling strategies should be implemented to have a high level of confidence that the pigs are negative and/or not shedding. Individual testing of pigs should be done if the veterinarian is particularly interested in having negative pigs since it has been shown that pigs can become persistently infected, harbor the virus in the tonsils, and shed the virus after stressful situations[3].

What can we learn from this case?

Piglets can be exposed to SVA in the farrowing room and shed the virus for potentially up to 8 weeks after placement in a wean-to-finish barn. SVA shedding from weaned piglets born at later stages of an outbreak in a sow farm may be undetectable. However, further testing piglets born at later stages of the outbreak would be beneficial to better understand their SVA status, given that their processing fluids were positive.

SVA transmission and infection events may still happen in the wean-to-finish barn but may occur at low levels. However, this may not be the case for wean-to-finish barns that commingle piglets from different sow farms, especially if one or more sow farms are naïve to SVA.

Take home messages

1. Oral fluid samples were consecutively positive until 3 weeks post-placement in the group of weaned piglets born during the first week of the SVA outbreak, but shedding may have happened for up to 8 weeks.
2. SVA shedding from weaned piglets born at the later stages of the outbreak was not detected. However, further testing would be beneficial to better understand these animals' SVA status, given that their processing fluids were positive.
3. New infections characterized by the detection of viremic piglets in the second week post-placement were not detected.

References

1. Joshi LR, Fernandes MHV, Clement T, et al. Pathogenesis of senecavirus a infection in finishing pigs. *J Gen Virol*. 2016;97(12):3267-3279. doi:10.1099/jgv.0.000631

2. Maggioli MF, Lawson S, de Lima M, et al. Adaptive Immune Responses following Senecavirus A Infection in Pigs. *J Virol*. 2017;92(3):1-17. doi:10.1128/jvi.01717-17

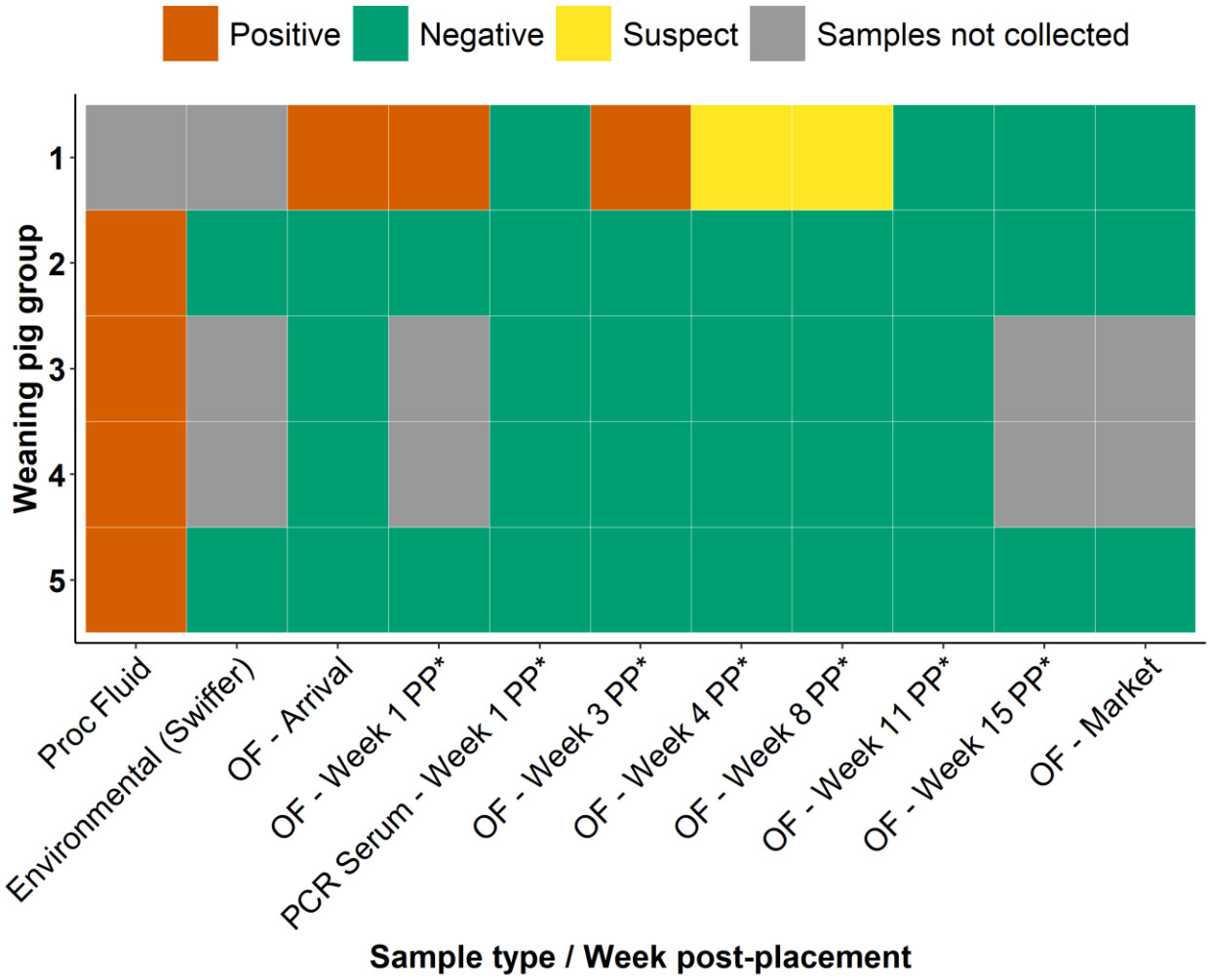
3. Maggioli MF, Fernandes MH V., Joshi LR, et al. Persistent Infection and Transmission of Senecavirus A from Carrier Sows to Contact Piglets. *J Virol*. 2019;93(21):1-19. doi:10.1128/jvi.00819-19

Table 1: Weaning pig groups farrowing/weaning dates, samplings, and other information.

Weaning groups and farrowing/weaning dates			Sampling and other activities in the wean-to-finish barns							
Group	SVA break date in the sow farm	Weaned/arrived in WF* barn	Week of arrival in WF* barn	1 week PP*	3 weeks PP*	4 weeks PP*	8 weeks PP*	11 weeks PP*	15 weeks PP*	1 week before market
1	Oct-29-2021	Nov-23-2021	OF	OF + blood	OF + Mhyo vax	OF	OF	OF	OF	OF
2	Oct-29-2021	Dec-17-2021	OF	OF + blood	OF + Mhyo vax	OF	OF	OF	OF	OF
3	Oct-29-2021	Dec-23-2021	OF	OF + blood	OF + Mhyo vax	OF	OF	OF	OF	OF
4	Oct-29-2021	Dec-30-2021	OF	OF + blood	OF + Mhyo vax	OF	OF	OF	OF	OF
5	Oct-29-2021	Jan-03-2022	OF	OF + blood	OF + Mhyo vax	OF	OF	OF	OF	OF

*WF: Wean-to-finish. *PP: Post-placement of pigs in the WF barns.

Figure 1: Heat-map chart from SVA rRT-PCR testing from each group of weaning pigs.



*PP: Week post-placement in the wean-to-finish barn.

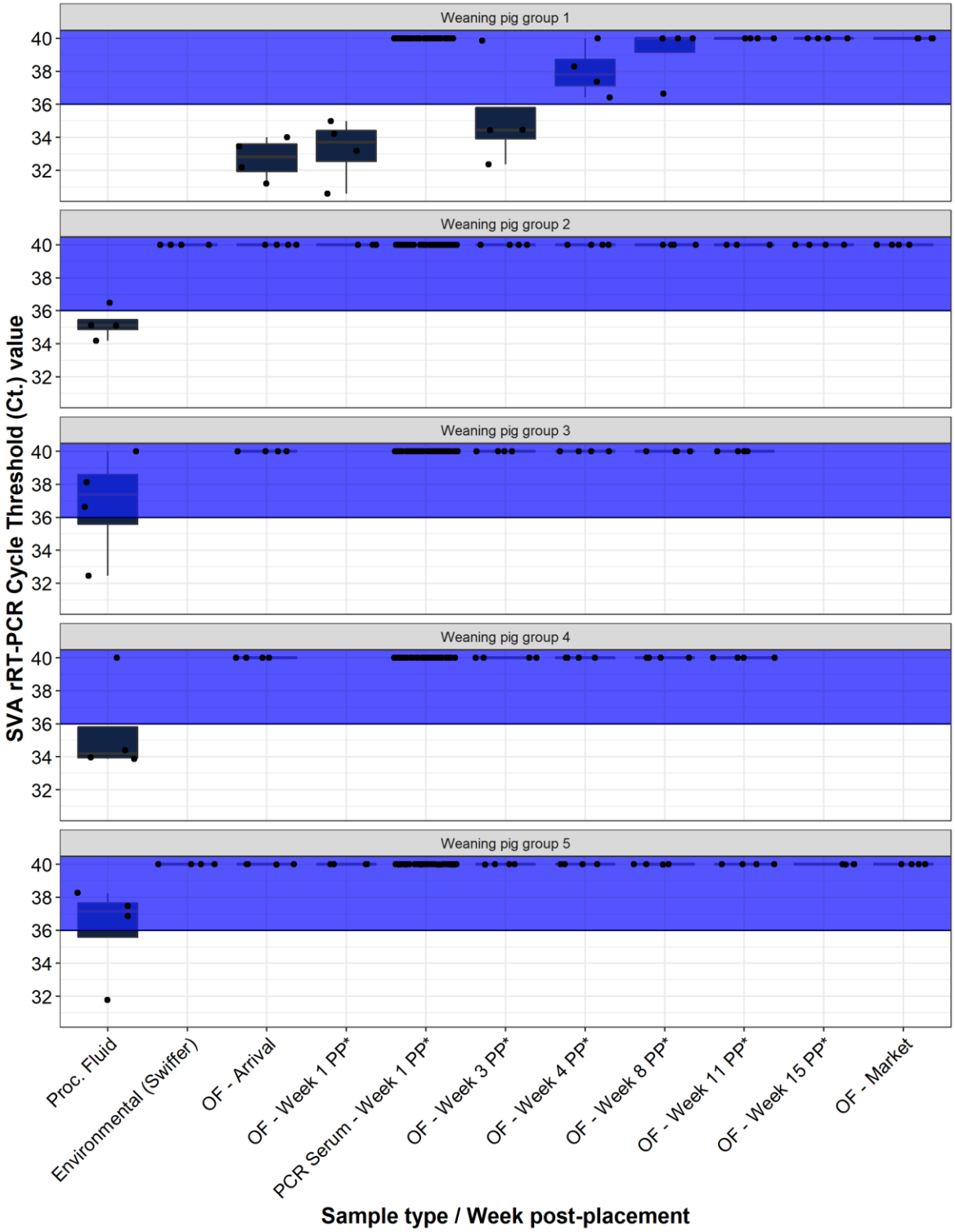


Figure 2: SVA rRT-PCR testing results from each group of weaning pigs by sample type and week post-placement in the wean-to-finish barn. Ct values inside light-shaded blue area (Ct >=36) represent suspect results. Ct values <36 are considered positive and negative when equal to 40.