Title: Assessing time to negative processing fluids in breeding herds after a Senecavirus A outbreak

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Statement of the problem

Senecavirus A (SVA) has been responsible for a rampant increase in the number of foreign animal disease investigations in the United States. Neonatal mortality and diarrhea have been associated with SVA infection in sow herds in different countries, but the pathogenesis and epidemiology of this disease is still unclear. Currently, swine practitioners have virtually no information about the presence of SVA in processing fluids in sow farms during an outbreak, and very little is known about its transmission within the farrowing room. Characterizing SVA detection in processing fluids over-time after an SVA outbreak has the potential to enable the industry to better control this disease by creating a system for time-to-stability estimation; similar to what is used with porcine reproductive and respiratory syndrome virus (PRRSV).

In addition, the role of heat-check boars on the persistence of the disease in a breeding herd after an outbreak is also unknown. Due to the prolonged period of time some of these animals remain in breeding herds, they might have some responsibility in the persistence of SVA and reoccurrence of outbreaks. Lastly, despite some farms reporting high piglet mortality during outbreaks, SVA-related production losses have not yet been properly characterized.

Objectives

- Estimate the time to negative processing after an SVA outbreak
- Assess the role of heat-check boars in the perpetuation, persistence and transmission of SVA within a farm
- Estimate the production losses associated with an SVA outbreak

Data collected and laboratory testing

A total of 310 processing fluids from 10 different sow farms were tested by SVA RT-qPCR. Seven farms belonged to one production system, while another production system and two veterinary clinics participated in the study with one sow farm each. One sow farm had the SVA outbreak detected in June/2019, while five farms had the SVA outbreaks detected in August/2020, two farms in September/2020, one in October/2020 and another in November/2020 (Table 1). The farm that had the SVA outbreak in June/2019 (Farm 1) was part of a preliminary study, but had its data incorporated to this report. The SVA outbreak date was defined as the date when clinical signs were noticed and reportable vesicular diseases such as FMD were ruled out.

Processing fluid samples were tested at different time-points during the study (e.g.weeks), before and after the SVA outbreak detection in each farm. Participating companies shipped the samples at different time-points and intervals, which were received, processed, and tested by the University of Minnesota (UMN) Veterinary Diagnostic Laboratory (VDL). In average, the farms in this study had processing fluids being tested from 2.7 weeks before and 19.1 weeks after SVA outbreak detection. Detailed information about each tested farm is shown in Table 1.

Only one farm (Farm 2) agreed to collect semen samples from the heat check boars, at weeks 7 and 18 after the outbreak.

Classification of SVA status in each sampled week

Every farm was classified as either positive or negative based on the weekly processing fluids results. Even though samples with SVA RT-qPCR Ct values ranging between 36 and 40 are usually considered as being suspects for diagnostic purposes, such samples were considered as positives in this study. Due to the dilution effect and loss of sensitivity due to the pooling of litters in processing fluids samples, suspect samples should be treated as positives since SVA could be present at a lower prevalence. Therefore, at any given sampling week a farm was considered to be positive if at least one processing fluids sample had a Ct value below 40.

Significant findings and recommendations

Due to the voluntary nature of the project and different logistic challenges between farms, the outbreak dates, number of samples and the sampling frequency and timelines were not equal among all farms.

The follow-up time in all farms ranged from 16 to 30 weeks, with an average follow-up of 22.5 weeks (Table 2). We were able to test PF samples collected before the outbreak detection in some farms, as seen in table 3.

Some farms were SVA positive by PF even before the clinical signs of SVA were detected, with the earliest detection of SVA RNA in PF up to three weeks before the outbreak (Table 3). The average number of weeks after the outbreak where at least one processing fluid was positive is 11.8, with a standard deviation of 5.2 (Table 2).

It is important to notice that one farm (Farm 2) still had a SVA-positive week at 21 weeks after outbreak detection. However, Farm 2 is the only farm that reported attempting mass SVA exposure 8 weeks after outbreak detection together with herd closure, aiming to eliminate SVA from the farm. It is likely that exposing animals in the farm to SVA increased the SVA transmission events, resulting in an increased detection of SVA in PF over time. Briefly, vesicular fluid from affected animals was collected and mixed with a phosphate-buffered saline (PBS) solution. This SVA/inoculum solution was then frozen and had an aliquot sent to the UMN VDL for RT-qPCR testing, which had a positive result with a Ct value of 17. The SVA solution was then retrieved, thawed, and 0.1mL was used to inoculate animals via the intranasal route. All pigs that did not present blisters or did not share the same pen space with a blister-affected animal was inoculated with this solution intranasally. Gilts were also randomly exposed to the remaining SVA solution by spraying.

At this point, it is unclear the number of consecutive PCR negative weeks needed to have some level of confidence that no weaned piglets are SVA positive. However, as shown in tables 2 and 3, most farms have a varying number of consecutive negative weeks between positive results, ranging from 1 to 10 weeks. Caution should be taken if the weaning of consistently SVA negative piglets is desired as the number of consecutive negative weeks needed to consider the sow farm as stable requires more investigation.

The number of followed weeks with negative results, after the last positive week, also varied. It has ranged from 2 weeks in two farms (Farms 2 and 7), to 14 weeks in Farm 1 and 18 weeks in Farm 4 (Table 3). However, Farm 4 had very atypical results, where SVA RNA was last detected at week 1 after the outbreak, which could be due to previous herd immunity acquired from a previous SVA outbreak. Also, both farms were not able to test weekly PF samples through the entire follow-up time, as seen in table 3, which could have changed this number if all weeks were tested without interruptions. Overall, all farms had an average of 7.3 consecutive negative weeks by PF after the last positive, which might be too few negative weeks if weaning of SVA negative piglets is highly desired.

It is currently unknown how the dilution effect due to litter aggregation and pooling changes the sensitivity of SVA RT-qPCR in PF. Further investigation should be performed to assess this issue, especially in later stages of SVA infection in a farm, as the proportion of positive litters are expected to decrease.

Heat check boars can also be a source of SVA infection to naïve gilts and sows. In the first semen collection at week 7 after the outbreak in one farm, 4 out of 9 boars had semen Ct values below 36 (positive), 3 out of 9 had Ct values above 36 (suspect) and 2 out of 9 boars had PCR negative semen. In the second semen collection time-point at week 18 after the outbreak, only 1 out of 16 boars had a Ct value above 36 (suspect). The boar with the suspect semen in the second time-point was euthanized at week 22, and the farm was asked to collect and ship tissue samples from the tonsils of the soft palate and testicles to the UMN VDL for PCR testing. Surprisingly, both the tonsil and testicle samples were positive for SVA, with Ct values of 30.5 and 17.8 respectively. These incredibly low Ct values, especially from the testicular sample, indicate that heat-check boars might act as carriers of SVA after an outbreak in a sow farm. More studies

should be performed to assess their role in the persistence and transmission of SVA to incoming naïve animals.

Unfortunately, only farm 1 provided weekly production data from a period ranging 52 weeks before and up to 16 weeks after detection of SVA clinical signs. The average pre-weaning mortality (PWM) from this period was 14.3%, for all cohorts of piglets weaned. The PWM at the week of SVA detection was 9.1%, which quickly increased in the next three weeks as SVA affected cohorts of piglets started being weaned. PWM mortality increased to 18.1% and 23% at weeks 1 and 2 after outbreak, and peaked at the third week with 42.7%. Afterwards, PWM decreased quickly to 17% at week 4, and 15.2% at week 5. Data pertaining PWM in farm 1 is shown in figure 1. We will continue to request production data.

How will these findings assist the practicing veterinarian?

Understanding the within-herd epidemiology of this disease can lead the industry to implement aggressive programs for elimination at a system level. This project is providing novel information about SVA infection dynamics, providing the first blocks for the construction of said elimination programs.

What we can learn from this case?

The data being generated in this study shows there is a large variation among farms when it comes to time-to-negative processing fluids. If a practicing veterinarian is extremely interested in weaning consistently SVA-negative piglets after an outbreak, s/he could initially monitor the farm with processing fluids until 10 consecutive PCR-negative weeks are accumulated. Afterwards, other sampling strategies and techniques might be needed.

Another important finding is the SVA positive testicle from a heat-check boar at 22 weeks after the outbreak. Practicing veterinarians should keep this in mind when aiming to control SVA in sow farms, as these animals might be acting as carriers of SVA to incoming naïve gilts over time.

Take home messages

- 1. SVA transmission within the farrowing room might be occurring during the first 21 weeks after the outbreak.
- 2. SVA might still be present in processing fluids after 10 consecutive negative weeks.
- 3. Heat-check boars may act as SVA carriers over time and infect incoming naïve animals.

Farm ID	Herd Size (n° of sows)	Month of SVA outbreak	N° of collected samples	N° of weeks followed before outbreak	N° of weeks followed after outbreak	Follow-up time (n° of weeks)	Average n° of samples per followed week
1	6,000	Jun-19	85	3	26	30	2.8
2	6,000	Sep-20	64	6	23	30	2.1
3	2,000	Aug-20	22	2	19	22	1.0
4	2,000	Aug-20	19	2	19	22	0.9
5	2,000	Aug-20	22	3	18	22	1.0
6	2,000	Aug-20	22	2	19	22	1.0
7	2,000	Aug-20	21	3	17	21	1.0
8	2,000	Sep-20	22	5	16	22	1.0
9	2,000	Oct-20	18	1	16	18	1.0
10	2,600	Nov-20	15	0	18	16	0.9*
TOTAL		_	310	_		_	
AVERAGE	—	—	—	2.7	19.1	22.5	1.3

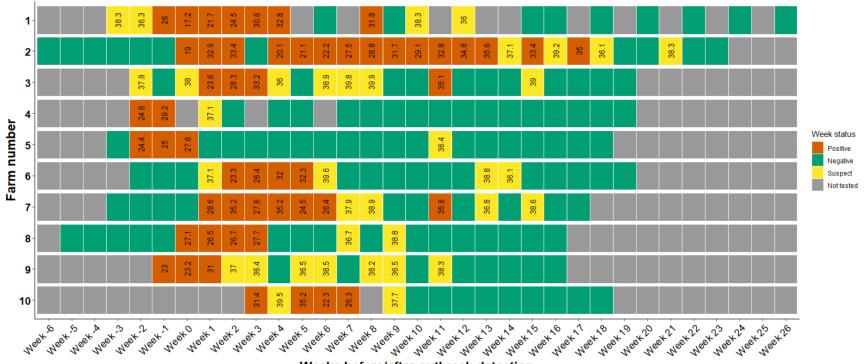
Table 1: SVA affected farms characteristics and information on the number of processing fluid (PF) samples and weeks tested.

*Farm 10 had no PF submitted/tested in one of the weeks after the outbreak, as depicted in table 3.

Table 2: Summary statistics of the number of followed weeks and SVA status in processing fluids over time, from 10 sampled sow farms.

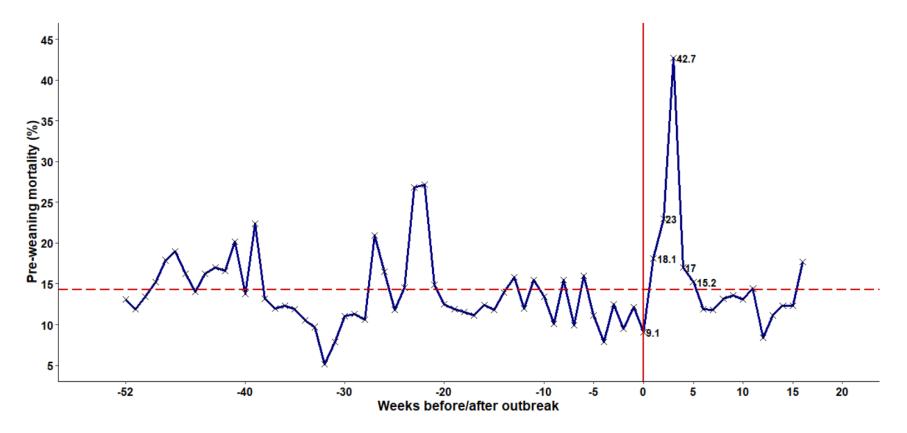
Statistic	Minimum value	First Quartile	Median	Third Quartile	Maximum value	Average	Standard Deviation
Total follow-up time in weeks	16	21.2	22	22	30	22.5	4.5
N° of followed weeks before outbreak detection	0	2	2.5	3	6	2.7	1.8
N° of followed weeks after outbreak detection	16	17.2	18.5	19	26	19.1	3.1
Last positive week after outbreak detection	1	9.5	11.5	14.7	21	11.8	5.2
Number of negative weeks between positive weeks	0	1.2	2	3	10	2.9	3
Number of negative weeks after last positive week	2	4.2	6	8.5	18	7.3	5.2





Weeks before/after outbreak detection

Figure 1: Pre-weaning mortality (PWM %), from cohorts of piglets weaned before and after SVA outbreak detection in Farm 1.



Horizontal dashed red line = average PWM throughout the whole period (14.3%).

Vertical solid red line = moment of outbreak detection (week 0).