

RAPID COMMUNICATION

Systematic Epidemiological Investigations of Cases of Senecavirus A in US Swine Breeding Herds

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Keywords:

Senecavirus A; Seneca Valley virus; vesicular disease; swine; epidemiology; investigation

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Received for publication May 11, 2016

doi:10.1111/tbed.12598

Summary

Epidemiological investigations were conducted on a case series of six Senecavirus A (SVA)-affected breeding herds in the United States to determine potential routes of introduction and enhance the swine industry's knowledge of SVA's clinical presentation and spread. Each SVA-affected herd was evaluated using a standard form to ensure that all relevant data were collected. The form was used to guide a detailed discussion about the clinical presentation of SVA and risk events that occurred in the 4 weeks prior to the first observation of clinical signs with the herd veterinarian and farm personnel. Each event was then subjectively assigned a risk level of low, medium or high likelihood for SVA introduction by the investigation team. The clinical presentation of SVA varied by case. All SVA-affected herds (six of six) reported increases in pre-weaning mortality and sow anorexia. Vesicular lesions were observed in four of six herds, and mild-to-moderate neonatal diarrhoea was observed in three of six herds. No gross anatomic or histologic lesions were observed in neonatal pigs that tested positive for SVA via PCR. Multiple potential routes of introduction were identified. Events subjectively rated as high risk for SVA introduction were on-farm employee entry (six of six), carcass disposal (four of six), cull sow removal (three of six) and breeding replacement entry (two of six). Non-swine domestic animals, rodents, other visitors, repairs outside swine barns, feed delivery, weaned pig removal and semen entry were assigned a high risk level in one of six herds. Cases occurred in breeding herds of all sizes with variable biosecurity in both swine dense and swine sparse areas.

Introduction

The US swine industry is susceptible to emerging and transboundary infectious diseases as evidenced by the 2005 introduction of porcine circovirus type 2, the 2013 introduction of porcine epidemic diarrhoea virus and, now, the re-emergence of Senecavirus A (SVA). Formerly known as Seneca Valley virus, SVA is a non-enveloped, single-stranded RNA virus in the family *Picornaviridae* (Adams et al., 2015) initially identified in 2002 as a cell culture contaminant (Hales et al., 2008). Isolates of SVA were recovered from pigs in the United States sporadically since 1988 (Knowles et al., 2006). An association between SVA and vesicular lesions in swine was described during 2007 in

Canada (Pasma et al., 2008), 2010 in the United States (Singh et al., 2012) and 2014 in Brazil (Leme et al., 2015; Linhares, 2015; Linhares et al., 2015; Vannucci et al., 2015a,b). More recently, acute losses in neonatal pigs have been associated with SVA (Linhares, 2015; Linhares et al., 2015; Vannucci et al., 2015a,b).

During late summer 2015, the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) experienced an increased incidence of SVA-positive swine cases reporting vesicular lesions. Between July 2015 and April 2016, 155 swine submissions at the ISU VDL had one or more samples test positive for SVA. Not all of the submissions were new cases, a minority may have originated from the same herd (J. Kraft, ISU VDL, personal communication, 2016).

SVA was first identified in exhibition swine in Iowa in July of 2015 and was subsequently identified in commercial finishers and breeding herds in nine states (Rademacher et al., 2016). The increased incidence of SVA-positive submissions reporting vesicular lesions caused concern because SVA is clinically indistinguishable from other vesicular diseases caused by swine vesicular disease virus, vesicular stomatitis virus, vesicular exanthema of swine virus and foot-and-mouth disease virus (FMDV). Swine veterinarians in the United States are required to report herds exhibiting vesicular disease to State or Federal animal health officials immediately. A foreign animal disease (FAD) investigation, conducted by the State or Federal animal health official according to the United States Department of Agriculture's Veterinary Services Guidance Document 7406.2, must be performed to ensure that the vesicular lesions were not caused by a trade impacting FAD (United States Department of Agriculture, 2016). After a FAD is ruled out, the case is handled by the herd's veterinarian and normal diagnostic laboratory.

To date, the swine industry lacks scientific information on the transmission of SVA. The objective of this study was to enhance the swine industry's knowledge of SVA's clinical presentation and spread by investigating SVA-affected breeding herds in a timely, efficient and uniform manner.

Materials and Methods

Epidemiological investigations were conducted on a case series of six Senecavirus A (SVA)-affected breeding herds in the United States from July to October 2015.

Criteria for enrolment

A case involved a single SVA-affected herd selected for epidemiological investigation based on the following criteria: (i) SVA was detected via polymerase chain reaction (PCR) from samples submitted to the ISU VDL, (ii) SVA-positive samples were from a farrow-to-wean, farrow-to-feeder or farrow-to-finish premises, and (iii) the herd veterinarian and farm personnel were willing to participate. Due to constraints on the number of investigations that could be performed, the investigations were limited to one per production system to avoid overrepresentation of a single production system. The Swine Health Information Center (SHIC), a US organization focused on research intended to reduce the impact of future swine disease threats (www.swinehealth.org), was notified that an investigation on the breeding herd was scheduled (information identifying the farm was not disclosed) to release funding for the investigation.

SVA investigation form

Each SVA-positive herd was evaluated using a standard form to ensure that all relevant data were collected. The form was modified from one developed and validated through the porcine reproductive and respiratory syndrome (PRRS) Outbreak Investigation Program, funded by the Iowa Pork Producers Association (Canon et al., 2015a, b). Data about the herd, premises, clinical presentation, diagnostics, and a comprehensive set of risk events and their associated carrying agents were collected. Carrying agents included anything that may be infected or contaminated with the virus. For example, the carrying agents commonly associated with semen entry include the semen, packaging, and the vehicle and driver delivering the semen. The risk events were organized into the following categories: (i) swine movement (semen entry, breeding replacement entry, cull sow removal and weaned pig removal); (ii) vehicles/deliveries (carcass disposal, feed delivery, propane/fuel delivery, garbage collection, new tools and supplies, and tools and supplies transferred from other swine premises); (iii) people movement (on-farm employees, repair inside/outside barns and other visitors); (iv) manure removal; (v) wildlife and non-swine domestic animals; and (vi) air/water entry. The 'SVA Investigation Form' is available upon request from the corresponding author.

SVA epidemiological investigation

The purpose of the SVA epidemiological investigation was to assess the clinical presentation of SVA, determine possible routes of introduction into the breeding herd and identify gaps in the farm's biosecurity. The purpose was not to rule out a FAD infection; therefore, the investigation team began work after SVA was diagnosed from samples submitted to the ISU VDL and did not have a role in any FAD investigations. The investigation coordinator, a research associate at Iowa State University's College of Veterinary Medicine (ISU CVM) with a background in swine production and research, communicated with the herd veterinarian to pre-populate sections of the 'SVA Investigation Form' and scheduled the investigation. Meetings were scheduled within 28 days of the first observation of clinical signs and occurred either at the farm or at an off-site location. The investigation facilitator, a veterinarian at ISU CVM with expertise in swine health, biosecurity, and epidemiology, and coordinator deployed for the investigation. The facilitator and coordinator met with the herd veterinarian and pertinent farm personnel. All investigations were performed by the same coordinator and facilitator.

The 'SVA Investigation Form' was used to guide a detailed discussion about the case, and risk events that

occurred in the 4 weeks prior to the date clinical signs were first observed by on-farm personnel. A retrospective 4-week investigation period was selected to provide a large margin of error around the exact date when SVA entered the farm as the observation of clinical signs sometimes lags the expression of clinical signs in a breeding herd and the incubation period for SVA was unknown. On average, the investigation was completed after 3 h of open-ended discussion (minimum of 2 h, maximum of 4 h). Upon completion of the investigation, the investigation coordinator composed a comprehensive summary report. The goal of the report was to illustrate to the herd veterinarian and swine producer where the largest gaps in their biosecurity programme were during that period of time, point out which gaps most likely resulted in virus entrance and provide guidance on improving their biosecurity programme with the hopes of preventing a future disease outbreak. The summary was returned to the herd veterinarian within 14 days of the investigation.

A risk level of low, medium or high was subjectively assigned by the investigation facilitator and coordinator to each event as a means to focus the producer's attention on events where large gaps in biosecurity were present. The subjective assessment was based on: (i) frequency of the event, (ii) likelihood that one or more carrying agents associated with the event were contaminated or infected with SVA on arrival to the premises and (iii) likelihood that infectious SVA was transmitted from the carrying agent to swine in the breeding herd. The risk levels for each event were determined by critically assessing all observations surrounding each event. Events that occurred more often inherently carried more risk as there were more opportunities for SVA introduction with that event. However, all three factors were considered jointly when assessing the risk of an event. For example, an event that occurred frequently may have been assigned a risk level of low because the biosecurity practices in place suggested a very low likelihood any of the carrying agents associated with the event were contaminated or infected on arrival or it was very unlikely that SVA was transmitted from the carrying agent to swine in the breeding herd. To determine the likelihood that the carrying agents associated with each event were infected or contaminated with SVA on arrival, the investigation team assessed where the carrying agents were prior to entering the premises. The likelihood that the carrying agents arrived infected or contaminated with SVA increased if any of the carrying agents had a connection to another SVA-positive swine premises, or were recently at another swine premises, swine harvest facility or other swine-related entity that could have been harbouring the virus. Biosecurity practices in place to detect and mitigate or just mitigate a contaminated or infected carrying agent were also assessed. Assessment of the likelihood of SVA

being transmitted from an infected or contaminated carrying agent that entered the premises to animals in the herd was based on the biosecurity practices in place at the time of the outbreak. The strength of the evidence for labelling an event high risk varied by case. In some instances, there was strong evidence indicating that a particular event was most likely responsible for the outbreak. For example, the first clinical signs were expressed in animals that were hauled on a trailer known to have been contaminated or there was a carrying agent, such as an employee, known to have been in contact with another SVA-positive premises. In other instances, the evidence was less clear, but observations such as the high frequency of the event, evidence that carrying agents contacted other swine premises or swine harvest facilities prior to entering the premises, lack of biosecurity practices to mitigate the contamination and lack of biosecurity protocols at the farm to prevent virus transmission from the carrying agent to swine in the herd circumstantially indicated that those events were higher risk than others for the introduction of SVA.

Results and Discussion

Enrolled breeding herd characteristics

Six SVA epidemiological investigations were completed. Investigations were conducted on a farrow-to-finish herd in Iowa (1) and farrow-to-wean herds in Illinois (1), Iowa (1), Minnesota (1) and Nebraska (2) (Table 1). Three herds were located in swine sparse areas with less than five other swine premises within a 5-mile radius. One herd was located in a moderately swine dense area with five to nine other swine premises within a 5-mile radius. Two herds were located in swine dense areas with more than 10 other swine premises within a 5-mile radius.

Clinical presentation

The clinical presentation of SVA for each case varied widely. Increased pre-weaning mortality (PWM) and neonatal ill thrift/lethargy in at least one farrowing room were reported in all cases (six of six) (Table 2). The increase in PWM was transient and resolved within 3 weeks. In one-half of the cases (three of six), a mild-to-moderate scour in neonatal pigs was reported. Interestingly, in all of the cases where neonatal pigs or tissue from neonatal pigs were submitted to the ISU VDL and were SVA-positive by PCR (five of six), no gross anatomic or histologic lesions of diagnostic significance were observed. Sows that were positive for SVA by PCR were anorexic in all of the cases (six of six). Vesicular lesions on the nasal and coronary band regions of breeding females were reported in four of six cases. During the acute phase of the outbreak, the incidence of vesicular lesions varied by herd,

Table 1. Characteristics of the six SVA-positive breeding herds investigated

Characteristic	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Location (US state)	Iowa	Illinois	Iowa	Minnesota	Nebraska	Nebraska
Stages of production	FTF	FTW	FTW	FTW	FTW	FTW
Farrowing system	Continuous	Continuous	Continuous	Continuous	5/4 Batch ^a	Continuous
Female housing system	Combination of individual and group housing	Individual housing	Individual housing	Individual housing (sows) Group housing (gilts)	Individual housing	Individual housing
Number of breeding females	300	2250	4300	850	623	990
Number of employees	3	5	13	5	4	3
Ventilation	Combination natural and mechanical – not filtered	Mechanical – not filtered	Mechanical – not filtered	Mechanical – not filtered	Combination natural and mechanical – not filtered	Combination natural and mechanical – not filtered
Dead disposal	Compost on-site	Shared compost pile off-site	Rendering	Shared compost pile off-site	Compost on-site	Compost on-site
Manure storage	Uncovered lagoon	Deep pits	Uncovered lagoon	Deep pits	Uncovered lagoon	Uncovered lagoon
Number of swine premises within 5 miles ^b	1	0	18	15	5	9
Number of days between outbreak and investigation	8	20	17	22	28	19

FTF, Farrow-to-finish; FTW, Farrow-to-wean.

^aFive groups of breeding females that farrow on 4 week intervals.

^bDetermined by an initial search for swine confinement like buildings using Google Earth; confirmed by the farm manger and herd veterinarian during the investigation meeting.

ranging from 10 to 70 per cent. On one farm, 90 per cent of the breeding females were severely lame. The clinical signs reported above were provided by the herd veterinarian and farm manager based on their production records.

The clinical signs observed in these six herds were consistent with the vesicular disease and epidemic transient neonatal losses reported in Brazil during 2014 where vesicular lesions on breeding females and acute neonatal death, lethargy and diarrhoea were associated with Senecavirus A after diagnostic evidence ruled out infections of FMDV, swine vesicular disease virus, vesicular stomatitis virus and vesicular exanthema of swine virus (Linhares, 2015; Linhares et al., 2015; Vannucci et al., 2015a,b). Consistent with the new cases reported here, lesions were rarely (2 per cent) observed in neonatal pigs from Brazilian SVA-affected herds (Linhares, 2015). Historically, SVA in North America was only associated with vesicular disease in adult swine (Pasma et al., 2008; Singh et al., 2012). Prior to the Brazilian cases in 2014 and these US cases in 2015, there was no described or known association of SVA with neonatal mortality, diarrhoea or ill thrift. The variety of clinical presentations across the six SVA-positive breeding herds investigated in this study, the recent cases in Brazil, and historical cases in the United States and Canada, suggests that other factors, such as co-infections, housing, environment

and a stress event, might contribute to the specific clinical signs observed in an affected herd.

Frequency of risk events

The frequency of risk events varied greatly between herds and depended on the size of the farm and type of farrowing system (weekly versus batch farrowing) used. Farms with more breeding females and more employees had more risk events during the 4-week investigation period. The total number of risk events per farm ranged from 132 to 441 events (Table 3). Events that occurred more than 10 times on a single farm during the investigation period included on-farm employee entry (six of six), feed delivery (three of six), carcass disposal (two of six), repairs outside swine barns (two of six), semen entry (two of six) and weaned pig removal (one of six).

Subjective assessment of risk

Events assigned a high risk level for SVA introduction by the investigation team were on-farm employee entry (four of six), carcass disposal (four of six), cull sow removal (three of six) and breeding replacement entry (two of six). Non-swine domestic animals, rodents, other visitors,

Table 2. Clinical signs associated with each SVA case as described by farm personnel at the time of epidemiological investigation

Clinical sign	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Increase in pre-weaning mortality	Yes (avg. of 28.51% per litter farrowed)	Yes (variable ranged from 20–70% per litter)	Yes (>50% in affected litters)	Yes (only in 1 farrowing room)	Yes	Yes (avg. of 15% per litter farrowed)
Duration of increased pre-weaning mortality	~2 weeks	~3 weeks	~2 weeks	~1.5 weeks	N/A	~3 weeks
Neonatal ill thrift/lethargy	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)
Neonatal diarrhoea	Yes (all pigs in affected litters)	Yes (all pigs in affected litters)	No	No	Yes (some pigs in affected litters)	No
Gross and histologic lesions in neonates	No lesions observed ^a	No lesions observed ^a	No lesions observed ^a	N/A	No lesions observed ^a	No lesions observed ^a
Breeding female anorexia	Yes	Yes	No	Yes	Yes	Yes
Nasal vesicular lesions on breeding females	Yes (~70% incidence)	Yes (~10% incidence)	Yes (~40% incidence)	Yes (~70% incidence)	No	No
Coronary band and hoof vesicular lesions on breeding females	Yes (~50% incidence)	Yes (~10% incidence)	Yes (~40% incidence)	Yes (~70% incidence)	No	No
Breeding female lameness	Yes (~40% incidence)	No	No	Yes (>90% incidence)	No	No

Clinical signs reported during the acute phase of the outbreak were from descriptions given by on-farm personnel during the investigation meeting. Each of the six herds kept different records with varying level of detail; therefore, all measures are not available for all farms (indicated by N/A).

^aSVA-positive neonatal pigs or tissues from SVA-positive neonatal pigs did not exhibit any gross anatomic or histologic lesions of diagnostic significance when examined at the Iowa State University Veterinary Diagnostic Laboratory.

Table 3. Total number of risk events in each category for each farm investigated

Risk event category	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Swine movement	2	25	46	24	4	17
Vehicles/deliveries	29	28	46	42	6	15
People movement	101	145	343	116	126	191
Manure removal	0	0	6	1	0	0
Total number of risk events	132	198	441	183	136	223

This table represents the cumulative number of risk events that occurred within each category during the 4 weeks preceding the first observed clinical signs of SVA. Individual risk events in each category were as follows: swine movement (semen entry, breeding replacement entry, cull sow removal and weaned pig removal); vehicles/deliveries (dead disposal, feed delivery, propane/fuel delivery, garbage collection, new tools and supplies, and tools and supplies transferred from other swine premises); people movement (on-farm employee entry, repair personnel working inside/outside barns and other visitors). The category of manure removal only contains the event for which it was named.

repairs outside swine barns, feed delivery, weaned pig removal and semen entry were assigned a high risk ranking in one of six herds (Fig. 1). Significant biosecurity gaps occurred. None of the farms (zero of six) used a bench entry system that required employees and visitors to sit on a bench, remove their outside footwear and enter the facility without stepping in the same area their footwear contacted as part of their people entry protocol. Five of six farms did not have written biosecurity protocols or formal biosecurity training for employees. Half of the farms (three of six) lacked shower-in-shower-out or downtime protocols. Lines of separation restricting truck drivers from entering the swine barns and on-farm employees from entering the livestock trailer were not followed during cull sow removal (four of six) and replacement gilt entry (two of six). Employees were allowed to re-enter barns after contacting compost piles (two of six) or using shared unwashed equipment to place carcasses in a compost pile that was shared with multiple swine premises (two of six).

Farms 1, 5 and 6 demonstrated atypically poor biosecurity, especially with regard to on-farm employee entry.

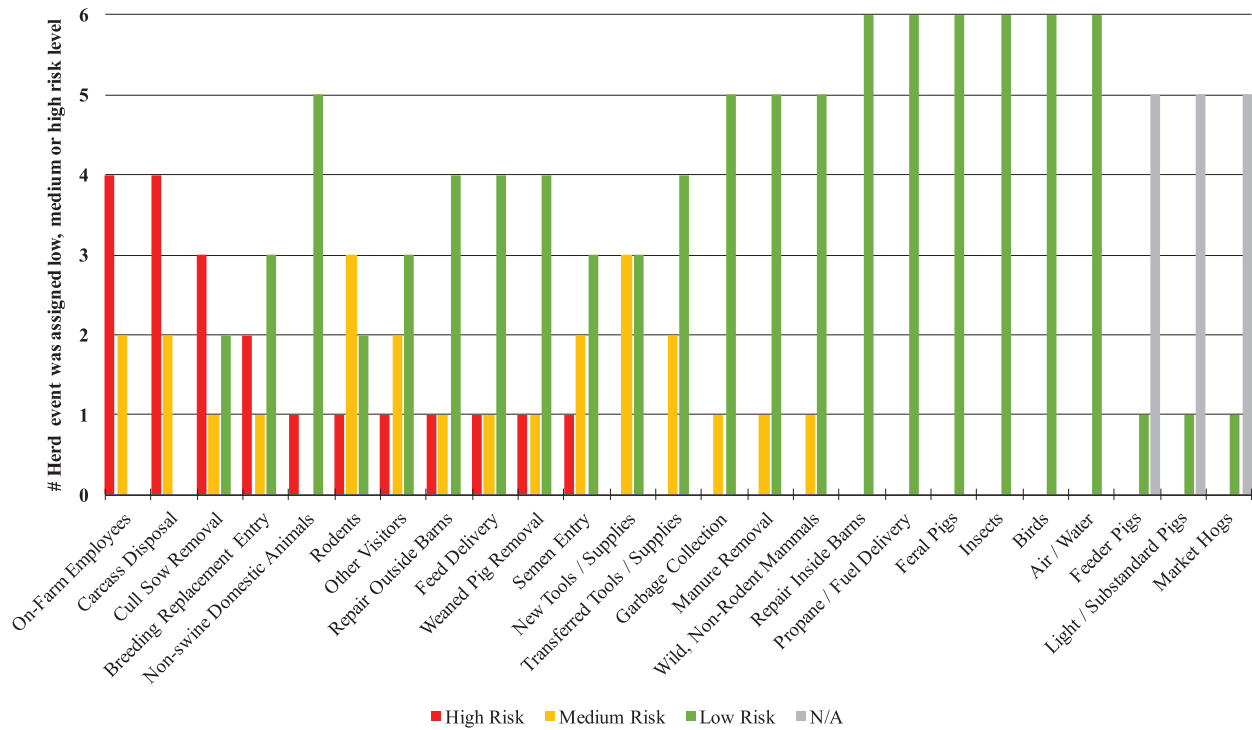


Fig. 1. Level of risk for SVA introduction assigned to each event across the six SVA-positive herds investigated. The investigation team subjectively assigned a risk level to each event after the investigation meeting based on the following criteria: (i) event frequency, (ii) the likelihood that a carrying agent was infected with SVA on arrival and (iii) the likelihood that SVA was transmitted from the carrying agent to a pig within the breeding herd. N/A indicates an event that did not apply to the herd (i.e. market hogs were not removed from farrow-to-wean farms, but were removed from farrow-to-finish farms).

Written biosecurity protocols or formal biosecurity training measures for employees were not in place at the time of the investigation. On-farm employees were not required to shower-in or shower-out of the facility, but were required to change clothing and boots prior to entry. Previous research on PRRSV, a single-stranded RNA virus, and FMDV, another picornavirus that causes vesicular lesions, but is not currently present in the United States, suggested that shower-in/shower-out protocols, or even changing clothing and boots and washing hands prior to entry, could prevent virus transmission from personnel to pigs (Otake et al., 2002; Amass et al., 2003, 2004). Farms 1, 5 and 6 did not require employees or other visitors to observe a period of downtime after contacting other swine. Early research on FMDV indicated that it could be detected in human nasal passages for up to 28 h (Sellers et al., 1970) and could be transmitted to naïve animals from humans (Sellers et al., 1971) leading to the current recommendation of a minimum of 48 h of downtime (no contact with other swine) after contacting swine infected with any pathogen. The need for this extended downtime is lessened if biosecurity

protocols are in place for personnel movement (Amass et al., 2003, 2004). As these farms lacked sanitation protocols for people entry, the absence of downtime requirements increased the risk of SVA transmission from on-farm employee entry. Farms 1, 5 and 6 allowed on-farm employees to exit the swine barns, perform tasks outside and then directly re-enter the barns without changing clothing or boots. Swine pathogens can be transferred to pigs from contaminated fomites (Pitkin et al., 2009) resulting in an elevated risk especially on farms 5 and 6 where employees directly re-entered after placing carcasses in an uncovered compost pile multiple times per day. The lack of biosecurity surrounding on-farm employee entry and the high frequency of the event were the basis for assigning on-farm employee entry a high risk level for the route of SVA introduction on farms 1, 5 and 6.

Farms 2 and 4 demonstrated a level of biosecurity typical of US breeding herds. Both required on-farm employees and visitors to shower-in and shower-out of the facility and practice downtime if they contacted other swine, but did not have formal biosecurity training or retraining

programmes. Farm 2 received breeding female replacements and semen from the same sources as another SVA-positive breeding herd, which declined to participate in this study, and delivered their cull sows to the same swine market. Farm 2's employees did not use a line of separation, which restricts them from entering the trailer and restricts the truck driver from entering the swine barn, when unloading gilts or loading cull sows. It is likely that the livestock trailers, gilts and/or truck drivers used at Farm 2 may have contacted vehicles, personnel or animals from the other SVA-positive breeding herd prior to entering Farm 2's premises. Thus, breeding replacement entry and cull sow removal were assigned a high risk level for introduction of SVA into Farm 2. Farm 4's usual replacement gilt trailer was inoperable during the investigated period. Gilts were delivered to Farm 4 on a substitute trailer that hauled cull sows to a swine market the previous day. On-farm employees did not comply with a line of separation for this load of gilts. The gilts hauled on this trailer exhibited the first clinical signs of SVA ≤ 4 days after delivery. This unusual event was considered most likely responsible for the introduction of SVA into Farm 4.

Farm 3 had better biosecurity practices than typical for US breeding herds, but also had a significantly higher frequency of events than the other five farms due to the size of the breeding herd. The large number of events (441) increased the number of opportunities for SVA introduction into Farm 3. On-farm employees were required to shower-in/shower-out and participate in biosecurity training and retraining programmes. Farm 3's manager worked at another SVA-positive swine premises during the investigation period. The manager was unaware that the other premises was SVA-positive at the time. Although the biosecurity protocols surrounding on-farm employee entry were good, compliance with these procedures was not 100%. This, combined with a farm manager that worked on an SVA-positive swine premises, was the basis for assigning on-farm employee entry a high risk level for SVA introduction into Farm 3.

While this case series provides valuable insight into the clinical presentation of SVA and risk events that occurred on these six farms prior to the SVA outbreak, the small sample size limits the repeatability and external validity of the case series. Additionally, data collection relied solely on the memories of the herd veterinarian and on-farm personnel. It is possible that some aspects of events that occurred during the investigation period were missed due to recall issues. To minimize the effects of recall issues, the investigation team performed five of six investigations within 3 weeks of the initial clinical signs and provided the herd veterinarian and on-farm personnel with a list of topics covered in the investigation 1 week prior to the actual investigation. All of the herd veterinarians and on-farm

personnel came to the investigation with physical records from the farm indicating the date the event happened and the personnel involved with the event. The subjective approach to risk assessment is also a limitation of this study. An objective approach would have been ideal, but in the absence of a significant research on the common methods of SVA transmission, a subjective approach based on previous epidemiological investigations performed on PRRSV by the same investigation coordinator and facilitator was the best method available at this time.

The SVA-affected herds investigated included small and large breeding herds with varying levels of biosecurity in both swine dense and swine sparse areas. The clinical presentation varied between cases. In these herds, SVA caused vesicular disease in breeding females as well as transient increases in neonatal mortality and ill thrift. Additional research is needed to assess why the clinical presentation may vary by case. Indirect transmission of SVA through the entry of contaminated people, livestock trailers or carcass removal equipment was subjectively assessed as the most likely routes of introduction. It is important to note that on-farm employee entry was assigned a medium (two of six) or high (four of six) risk level in all of the SVA-affected herds. To address this, the swine veterinary industry should improve communication with producers on the importance of biosecurity measures and provide encouragement to improve compliance with procedures currently in place to reduce the risk of virus transmission via on-farm employees in the event of other SVA, endemic or emerging disease outbreaks. Research should be conducted on the value of adding additional layers of biosecurity to people movement events to further mitigate this risk.

Acknowledgements

The authors would like to thank the Swine Health Information Center (SHIC) and the National Pork Board (NPB) for assistance in securing funding for this project, as well as the herd veterinarians, farm owners and farm personnel for their willingness to participate in the SVA epidemiological investigations.

Conflict of Interest

The authors declared no potential conflict of interest with respect to the research, authorship and/or publication of this article.

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