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Short communication

Experimental Seneca Valley virus infection in market-weight gilts

Alexandra Buckley[a], Vikas Kulshreshtha[a,1], Albert van Geelen[a], Nestor Montiel[a,2], Baoqing Guo[b], Kyoung-Jin Yoon[b], Kelly Lager[c,*]

[a] Oak Ridge Institute for Science and Education and National Animal Disease Center, Agriculture Research Service, U.S. Department of Agriculture, Ames, IA, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[c] Virus and Prion Research Unit, National Animal Disease Center, Agriculture Research Service, U.S. Department of Agriculture, Ames, IA, USA

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ABSTRACT

Seneca Valley virus (SVV) is a picornavirus that causes vesicular disease in swine. Since it is clinically indistinguishable from vesicular disease caused by food-and-mouth disease virus (FMDV), investigations must be performed to rule out this high consequence pathogen. A large portion of these investigations have involved market-weight swine at slaughter plants. The objective of this study was to describe acute infection dynamics of market-weight gilts (8 months of age) experimentally infected with SVV. At 0 days post inoculation (dpi) all gilts (n = 15) were given an intranasal SVV inoculation. Vesicular lesions on the coronary band were first observed on one or more feet by 2 dpi in 4 of the 15 gilts and in all by 5 dpi. Vesicles on the snout were observed in 6 of the 15 gilts beginning at 4 dpi. All gilts became viremic post challenge for about 7 days and developed anti-SVV neutralizing antibodies by 7 dpi. Most vesicular lesions were resolved by 14 dpi. Understanding the pathogenesis of SVV is critical in order to inform decisions that veterinarians and producers must make at the farm level to control this disease.

1. Introduction

Seneca Valley virus (SVV) is a non-enveloped positive-sense single-stranded RNA virus and the only member of the genus *Senecavirus* in the family *Picornaviridae* (Hales et al., 2008). First discovered as a cell culture contaminant, SVV was subsequently found sporadically in swine samples from the United States (US) dating back to the late 1980s (Knowles et al., 2006). Beginning in 2014, outbreaks of porcine idiopathic vesicular disease occurred in Brazil (Leme et al., 2015) followed by the US in 2015 with samples testing positive for SVV (Guo et al., 2016). Although previous efforts to reproduce disease with SVV had failed, growing evidence from the field suggested SVV was the etiology of the vesicular disease outbreaks (Knowles et al., 2006; Singh et al., 2012; Yang et al., 2012). This was confirmed with 2015 SVV isolates in weaned pigs (Chen et al., 2016), nursery pigs (Montiel et al., 2016), and growing pigs (Joshi et al., 2016a).

Since the vesicular disease caused by SVV is indistinguishable from foot-and-mouth disease virus (FMDV) in swine, in countries that are members of the World Organization for Animal Health (OIE), cases of vesicular disease in livestock must be investigated to rule out the presence of FMDV infection. Diagnostic investigation of vesicular disease may require extensive resources as found recently in the US when a dramatic number of foreign animal disease investigations at slaughter plants occurred (Hause et al., 2016), in each case SVV was the only known vesicular etiologic agent detected. Although these investigations are expensive to conduct, the temporary restriction on the movement of animals to slaughter and resulting disruption of market supply chains could be even more costly. To better understand the ecology and pathogenesis of SVV infection in swine destined for slaughter, the following study was conducted investigating the acute SVV infection in market-weight gilts.

2. Methods

Fifteen 8-month-old female white crossbred swine weighing approximately 150 kg were purchased from a commercial source in Wisconsin, US. Animals were clinically free of lesions and pre-challenge serum was negative for SVV neutralizing antibodies. These gilts were

* Corresponding author at: 1920 Dayton Avenue, P.O. Box 70, Ames, IA 50010, USA.
E-mail addresses: alexandra.buckley@usda.gov (A. Buckley), vikas.kulshreshtha09@gmail.com (V. Kulshreshtha), albert.vangeelen@usda.gov (A. van Geelen), nestor.a.montiel@aphis.usda.gov (N. Montiel), bqguo@iastate.edu (B. Guo), kyoon@iastate.edu (K.-J. Yoon), kelly.lager@usda.gov (K. Lager).
[1] Present address: Toxikon Corporation, Bedford, MA, USA.
[2] Present address: Avian Viruses Section, Diagnostic Virology Laboratory, National Veterinary Services Laboratories, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Ames, IA, USA.

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housed at the US Department of Agriculture's National Animal Disease Center in accordance with Institutional Animal Care and Use Committee protocol (ACUP 2867) and split into two groups for sample collection to reduce the potential stress of daily bleeding: Group A (n = 7) and Group B (n = 8). SVA15-41901SD was isolated on swine testicular (ST) cells from field samples of a case of vesicular disease in finishing swine (Guo et al., 2016; Zhang et al., 2015). Cell culture from passages 2 and 3 were combined and centrifuged to make the virus stock, which was diluted in MEM for the inoculum. All 15 gilts received 5 mL (1×10^7 $TCID_{50}$/mL) of inoculum intranasally on 0 days post inoculation (dpi), bled on 0, 17, and 33 dpi and checked daily for clinical signs of vesicular disease. Group A was bled on 1, 3, 5, 7, and 9 dpi, and Group B was bled on 2, 4, 6, 8, and 10 dpi. Serum samples and swabs of vesicles placed in 3 mL of MEM were stored at −80 °C for future testing. Clinical signs were scored with a 9 point system: each claw that displayed a vesicular lesion was given 1 point and a vesicle on the snout was assigned 1 point.

SVV RNA extraction and real-time quantitative RT-PCR testing was performed on serum samples and vesicle swabs as previously described with C_t values greater than 35 considered negative (Buckley et al., 2018). The primers and probe were designed to target a conserved region containing nucleotides 602–710 of the SVV genome. The forward primer sequence was 5′-TGCCTTGGATACTGCCTGATAG-3′, the reverse primer sequence was 5′-GGTGCCAGAGGCTGTATCG-3′ and the probe sequence was 5′-CGACGGCCTAGTCGGTCGGTT-3′. RNA copies were calculated based on a standard RNA transcript overlapping the target region.

In addition, a virus neutralization (VN) assay was completed using heat inactivated serum as previously described (Buckley et al., 2018). In the initial run, serum was serially diluted 1:4, ranging from 1:4 to 1:4096, and tested in quadruplicate. Samples that did not reach an end-point were repeated to obtain an end-point titer. Titers were reported at the highest dilution of serum for which cytopathic effect (CPE) was completely neutralized in 50% of the wells. A negative cutoff of ≤1:16 was established for the VN assay performed in this study and was based on unpublished VN results of pre-challenge pigs.

3. Results

At 2 dpi intact vesicular lesions and lesions that appeared to be ruptured vesicles were first observed on coronary bands of 4/15 gilts. The first snout vesicle was not detected until 4 dpi. Only 6 gilts (40%) developed snout lesions throughout the study, while all 15 gilts (100%) developed coronary band lesions on one or more feet by 5 dpi (Fig. 1). The median clinical score for the gilts was 3 with a range of 1–7. The caudolateral aspect of the lateral claw on the hind limbs was most commonly affected; however, cutaneous lesions were also seen on the coronary bands of both medial and lateral claws as well as dewclaws. Lesions typically began with a pale blanched area where the skin would slough off leaving an eroded/ulcerated area that would heal via epithelialization (Fig. 2). Two weeks after inoculation most snout and coronary band lesions had healed.

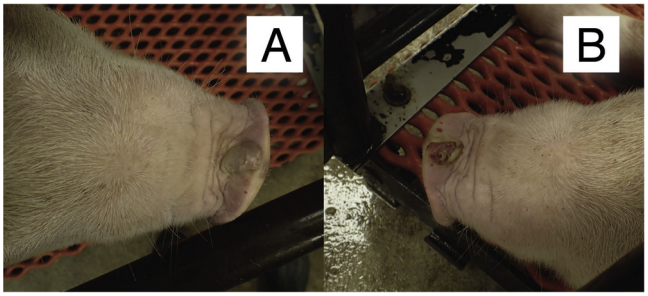

Fig. 1. Snout lesions in gilts after experimental inoculation of SVV. A) Intact snout vesicle on 6 dpi. B) Ruptured snout vesicle on 9 dpi.

Hemorrhages in the proximal to mid-hoof wall were observed in 12/15 animals and cracks in the hooves were observed on at least one claw of every gilt post inoculation (Fig. 3). The first area of hemorrhage appeared on one gilt on 3 dpi but most animals did not develop hemorrhage under the hoof walls until 8 dpi and later. Cracks first began to appear in hooves at 2 dpi and continued to develop in animals throughout the study. Intermittent mild lameness was observed starting on 2 dpi noticed as a reluctance to rise in some gilts. It was difficult to quantitate the extent of the lameness since a gilt may appear reluctant to rise one morning, but appear normal the rest of the study. The transitory mild lameness appeared to resolve within 7–10 days and did not affect their appetite.

At 1 dpi, 4/7 gilts in Group A were positive by PCR for SVV in serum. All pigs in Group A were positive for SVV RNA by 3 dpi, which was the peak of viremia with an average of 6.02×10^6 genomic copies (GC)/mL (Fig. 4). Only one pig in Group A was still positive at 9 dpi. All pigs in Group B were PCR positive for SVV at 2 dpi when peak viremia was observed with an average of 1.29×10^7 GC/mL. By 10 dpi only one pig in Group B was PCR positive for SVV in serum. In both groups, gilts remained positive for SVV RNA until around 7 dpi when the numbers of pigs with detectable viremia began to decline. Two gilts were PCR positive at 17 dpi but were not the same two that were SVV positive in serum at 9 and 10 dpi, respectively. All gilts were negative for SVV in serum on 0 and 33 dpi when the study ended. All fifteen gilts had at least one SVV-positive vesicle swab throughout the experiment with an average of 1.97×10^8 GC/mL, (range 1.54×10^4–1.17×10^9 GC/mL)

Serum from gilts was tested by VN assay to determine neutralizing antibody response to infection. VN titers from 0 dpi to 3 dpi were ≤ 1:16 for all animals (Table 1). Two gilts developed titers > 1:16 on 4 dpi (1:64) and on 5 dpi 5/7 animals had positive VN titers ranging from 1:256 to 1:1024. VN titers for all gilts ranged from 1:256 to 1:4096 through the remaining time points. Neutralizing antibody titers decreased for some animals between 17 dpi and 33 dpi.

4. Discussion

Prior to 2015, reproduction of clinical disease with SVV was unsuccessful (Bracht et al., 2016; Knowles et al., 2006; Yang et al., 2012) leaving in question the potential role of SVV in the etiology of a vesicular disease in swine. Recently, vesicular disease has been reproduced with 2015 US isolates in 3-week-old pigs (Chen et al., 2016), 9-week-old pigs (Montiel et al., 2016), and 4-month-old pigs (Joshi et al., 2016a). In the current study, vesicular disease was reproduced in 8-month-old gilts. All gilts developed lesions on the coronary bands after inoculation, while less animals developed snout lesions. The prevalence of snout lesions observed in this study was more than that reported in 9-week-old pigs with 12 pigs per treatment group (Montiel et al., 2016), but less than those reported in studies with smaller treatment groups using 3-week-old pigs (Chen et al., 2016) and 4-month-old pigs (Joshi et al., 2016a). This potential difference may be related to viral properties and/or host factors, though contemporary US isolates have been reported to share a 98–99% nucleotide identity (Joshi et al., 2016b). The distinction in lesion development between the snout and coronary band regions observed in this study with market-weight animals may be important since the coronary band region can be more difficult to thoroughly examine compared to the snout, especially in commercial swine farms with pigs reaching market weight. Therefore, lesions could be missed if observation is overly focused on the snout for vesicle development. In addition, lesions on the coronary bands were usually observed before vesicles were seen on any snouts. This timeline of vesicle development has also been reported in swine experimentally inoculated with FMDV (Stenfeldt et al., 2014).

This study and others have established that vesicular lesions on the coronary bands and snout have the highest concentration of SVV nucleic acid (Joshi et al., 2016a). Similarly, tissues from the coronary

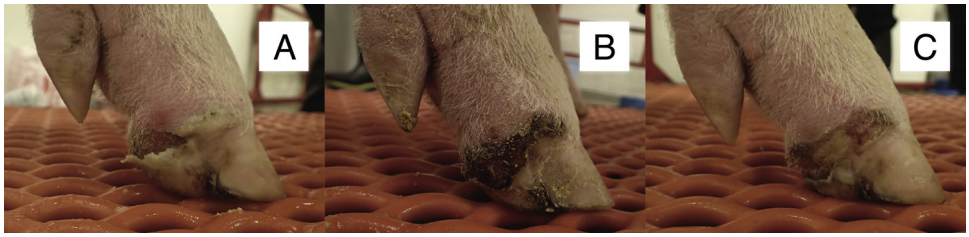

Fig. 2. Timeline of coronary band lesions in a gilt experimentally inoculated with SVV. A) Recently ruptured coronary band vesicle on 5 dpi. B) Erosive ruptured vesicle on 7 dpi. C) Healing vesicle on 9 dpi.

Fig. 3. Additional observations. A) Multiple areas of hemorrhage along the coronary band and the proximal to mid-hoof wall on 13 dpi. B) Resolving coronary band lesion with a crack in the hoof wall on 7 dpi.

band region have been reported to contain the highest quantity of FMDV viral RNA (Murphy et al., 2010; Stenfeldt et al., 2014). Additional observations in this study were nail bed hemorrhages and cracks in the hoof wall that were only noticed after SVV inoculation. However, there were no uninoculated animals for comparison, and these lesions may not be the direct result of SVV infection. Additional SVV studies could provide a better understanding of these lesions since hoof wall hemorrhages have also been associated with SVV outbreaks in the field (Dr. Rademacher, personal communication). Although the vesicular disease appears similar for SVV and what has been reported for FMDV, to our knowledge hoof cracks and hoof wall hemorrhages have not typically been associated with FMDV infection in swine.

Daily serum sample testing characterized the timeline and magnitude of viremia during the first ten days of infection with SVV in market-weight pigs. Viremia peaked at 2 and 3 dpi for Group B and A respectively and declined over the following week in the fifteen gilts. Viremia kinetics reported here are similar to those described by others after experimental inoculation with SVV (Chen et al., 2016; Joshi et al., 2016a; Maggioli et al., 2018) as well as those reported for pigs inoculated with FMDV (Alexandersen et al., 2001; Stenfeldt et al., 2016b). Two animals were PCR positive for SVV on 17 dpi but were not positive on their respective previous sample, suggesting, a small percentage of animals in a population may have an extended viremia that could be intermittently detected by PCR after exposure. Information on viremia kinetics is important to help veterinarians in the field make critical decisions such as timing of pig movements to manage and control the spread of SVV. This study has reinforced that the optimum time to collect serum samples is early in the infection process when lesions are first observed to improve the chances of making a positive SVV diagnosis with serum. Taken into consideration with the timeline of lesion development and healing, it is possible for animals with lesions to be negative for SVV in serum.

Neutralizing antibody titers in the gilts developed quickly, around 4–5 dpi, and peak neutralizing antibodies coincided with a reduced number of viremic animals, which has been previously reported in SVV infected animals (Joshi et al., 2016a; Maggioli et al., 2018). FMDV-neutralizing antibodies have also been measured in serum just before the clearance of viremia (Stenfeldt et al., 2016a). The VN results of gilt 723 during the first three days of infection provide an example supporting our negative cutoff value of $\leq$1:16, which may account for non-specific neutralization. The titer of gilt 723 was $<$ 4, 16, and 4 on 0, 1, and 3 dpi respectively. Further research will need to be performed to determine what virus neutralization titers confer protection and the duration of protective immunity after exposure to SVV.

Details about the acute infection kinetics of SVV in serum can provide valuable information to veterinarians and swine producers about the timeline of infection on the farm and epidemiology of this disease. Animals that are PCR positive for SVV in serum, but do not have neutralizing antibodies are most likely acutely infected, since multiple groups have reported neutralizing antibody development by 5 dpi (Joshi et al., 2016a; Maggioli et al., 2018; Montiel et al., 2016). If animals have detectable neutralizing antibodies but are not viremic, they are more likely to have been infected for at least one week.

5. Conclusion

A greater understanding of SVV pathogenesis can assist with better

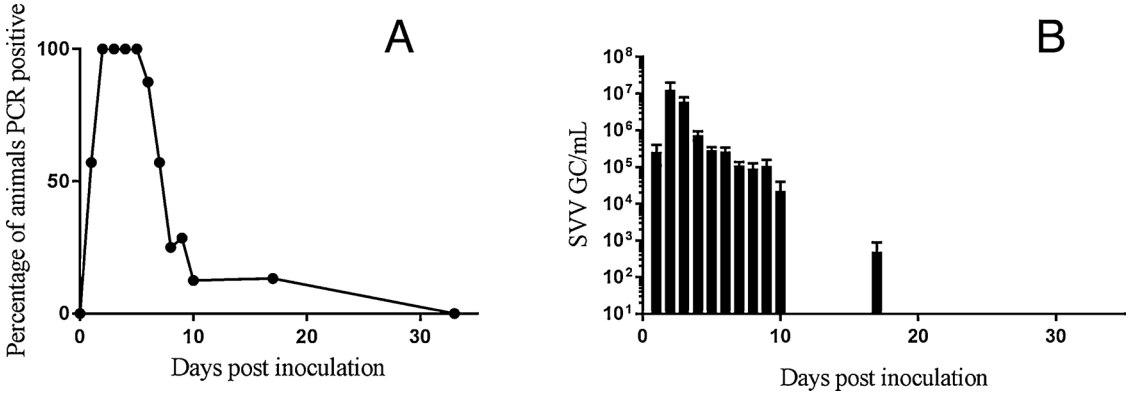

Fig. 4. Kinetics of SVV viremia in gilts after experimental infection. A) Percentage of SVV PCR positive gilts in serum at each bleeding time point. B) Mean genomic copies/mL of serum for each sample collection time point. Error bars represent the standard error of the mean.

Table 1
Virus neutralization assay results for each bleeding time point from 0 to 33 dpi.

	0	1	2	3	4	5	6	7	8	9	10	17	33
722	< 4	4	–	4	–	256	–	4096	–	4096	–	1024	256
723	< 4	16	–	4	–	16	–	1024	–	1024	–	1024	1024
724	4	4	–	4	–	256	–	4096	–	4096	–	1024	256
725	< 4	< 4	–	4	–	256	–	1024	–	1024	–	256	256
726	< 4	< 4	–	4	–	256	–	4096	–	1024	–	1024	1024
727	4	4	–	4	–	16	–	1024	–	4096	–	4096	1024
728	< 4	< 4	–	4	–	1024	–	4096	–	4096	–	256	256
729	4	–	< 4	–	4	–	1024	–	1024	–	1024	1024	256
730	< 4	–	< 4	–	4	–	256	–	1024	–	4096	1024	256
731	< 4	–	< 4	–	16	–	1024	–	4096	–	4096	1024	256
732	< 4	–	4	–	16	–	1024	–	4096	–	4096	1024	256
733	< 4	–	< 4	–	64	–	4096	–	1024	–	1024	256	256
734	4	–	4	–	16	–	1024	–	4096	–	4096	1024	1024
735	4	–	4	–	16	–	1024	–	1024	–	1024	256	256
736	< 4	–	4	–	64	–	4096	–	4096	–	1024	1024	256

– Not tested.

sample collection and faster diagnosis of this virus, as well as improved disease control and management measures in the swine industry. This study demonstrated that swabs of vesicular lesions have the greatest amount of viral nucleic acid, there is a short duration of viremia that can be detected by PCR, and animals quickly develop a neutralizing antibody response against SVV after infection.

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Author's contributions

AB, NM, VK, AVG, KJY, and KL were involved with conception and design of the study. AB, NM, VK, AVG performed the infection trial in pigs and collected all samples. BG and KJY contributed essential materials. AB performed laboratory testing of samples. AB and KL wrote the manuscript. All authors read, provided edits, and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicting interests.

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