Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

RAPID COMMUNICATION

Neonatal Mortality, Vesicular Lesions and Lameness Associated with Senecavirus A in a U.S. Sow Farm

P. Canning[1], A. Canon[1], J. L. Bates[1], K. Gerardy[2], D. C. L. Linhares[2], P. E. Piñeyro[2], K. J. Schwartz[2], K. J. Yoon[2], C. J. Rademacher[2], D. Holtkamp[2] and L. Karriker[1]

[1] Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[2] Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Keywords:
Seneca Valley virus; Senecavirus A; vesicular disease; lameness; swine; neonatal mortality

Correspondence:
L. Karriker. Swine Medicine Education Center, Iowa State University College of Veterinary Medicine, 2412 Lloyd Veterinary Medical Center, Ames, IA 50011, USA. Tel.: +515 294 2283; Fax: +515 294 1072;
E-mail: karriker@iastate.edu

Received for publication October 20, 2015

doi:10.1111/tbed.12516

Summary

A 300-sow farrow-to-finish swine operation in the United States experienced a sudden and severe increase in mortality in neonatal piglets with high morbidity followed by vesicular lesions on the snout and feet of adult females and males. Affected live piglets were submitted for diagnostic investigation. Samples tested polymerase chain reaction (PCR) negative for foot-and-mouth disease virus, porcine delta coronavirus, porcine epidemic diarrhoea virus, porcine rotavirus types A, B and C, transmissible gastroenteritis virus, and porcine reproductive and respiratory syndrome virus. Senecavirus A (SV-A) formerly known as Seneca Valley virus was detected by real-time reverse-transcription polymerase chain reaction (rRT-PCR) from serum, skin and faeces of piglets and from serum and faeces of sows. SV-A was isolated in cell culture from piglet samples. SV-A VP1 gene region sequencing from piglet tissues was also successful. A biosecurity and disease entry evaluation was conducted and identified potential biosecurity risks factors for the entry of new pathogens into the operation. This is the first case report in the United States associating SV-A with a clinical course of severe but transient neonatal morbidity and mortality followed by vesicular lesions in breeding stock animals. Veterinarians and animal caretakers must remain vigilant for vesicular foreign animal diseases and report suspicious clinical signs and lesions to state animal health authorities for diagnostic testing and further investigation.

Introduction

Senecavirus A (SV-A), formerly known as Seneca Valley virus, is the only species in the genus Senecavirus of the *Picornaviridae* family. SV-A is a single-stranded, non-enveloped RNA virus (Hales et al., 2008). First discovered as a cell culture contaminant during 2002, SV-A has drawn interest for its oncolytic properties and its use against neuroendocrine cancers in humans (Reddy et al., 2007; Hales et al., 2008).

Although its pathogenic role in swine has not been described, SV-A has been detected and, in some cases, isolated from pigs with cases of acute lameness, sudden neonatal death and vesicular lesions in North and South America (Amass et al., 2004; Pasma et al., 2008; Vannucci, 2015; Vannucci et al., 2015). During September 2014, Brazil experienced acute outbreaks of snout and coronary band vesicular lesions and sudden neonatal death loss, both of short duration, on sow farms (Vannucci et al., 2015). SV-A was identified by real-time reverse-transcription polymerase chain reaction (rRT-PCR) and by *in situ* hybridization in tissues and vesicles from affected piglets (Vannucci, 2015). SV-A has also been described as vesicular disease in pigs of all ages (preweaning, nursery, finishing and breeding stock) in Brazil on February 2015 (Leme et al., 2015). This report describes the first identification of SV-A in a disease outbreak manifested by lameness, vesicular disease and neonatal death on a farrowing-to-finish farm in the United States.

© 2016 Blackwell Verlag GmbH

Materials and Methods

Herd history and case presentation

The farm is a 300-sow, mixed parity, commercial, farrow-to-finish swine farm located in Iowa, USA. The site consists of a farrowing and nursery barn, gestation barn, grower barn and finishing barn. The west end of the finishing barn is used as overflow for gestation; therefore, gestating sows are housed adjacent to finishing hogs in this barn. The operation raises its own maternal line replacement gilts and has on-site terminal sires for artificial insemination. The farm is mechanically and naturally ventilated. None of the buildings on the site are filtered. A residence and feed mill are also on the same property.

A sudden increase in mortality among 1- to 5-day-old nursing piglets was first observed by the owner on 22 August 2015. Surviving piglets were weak, lethargic and having difficulty nursing. Rectal temperatures were not collected on these piglets.

The herd veterinarian was contacted on August 24. The owner reported eight of the ten litters born that week were affected with 75% mortality in five of eight affected litters. Historic pre-weaning mortality (PWM) for this farm was 14–18%. Pigs <7 days of age exhibited mild diarrhoea, ill thrift, anorexia and lethargy. Temperatures on piglets were not collected. Two of the sows with affected litters were anorexic but afebrile. The anorexia was attributed to recent farrowing. Sows were reported to be producing milk and did not have abnormal vaginal discharge, mastitis or lameness. Based on the clinical signs described by the producer, the clinical syndrome appeared to be affecting mainly neonatal piglets. The top differential diagnoses were porcine respiratory and reproductive virus (PRRSV) or porcine epidemic diarrhoea virus (PEDV). The farm was documented in February 2015 as PRRSV positive and unstable. There was no history of PEDV on this farm.

The producer was unable to collect serum from sows of affected litters, and the herd veterinarian was unable to collect samples on that day. As a result, eight affected live piglets and one recently dead piglet were submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) on 25 August 2015. Piglets were humanely euthanized and necropsied by the ISU VDL. Following the submission of these samples, the owner described skin lesions on the noses of two of the ten sows farrowed that week. Pictures of the lesions were taken and appended to the diagnostic case.

Herd evaluation

An on-site herd evaluation was conducted by the herd veterinarian on 31 August 2015, which was 9 days after clinical signs were first observed. Pigs from all operational stages including farrowing, gestation, nursery, grower and finisher were examined. Additional live piglets from affected litters (five) were submitted to the ISU VDL for diagnostic testing. Serum, faecal swabs, tonsil swabs, vesicular scrapings, vesicular fluid swabs and nasal swabs were collected from sows and piglets in the farrowing house and sows in the gestation building. All samples were submitted to the ISU VDL to confirm the presence of SV-A and to assist with the development of SV-A diagnostic assays.

On 11 September 2015, the herd veterinarian visited the farm to reassess clinical signs about 3 weeks after the initial outbreak. During this visit, a dead rat and semen from a clinically recovered boar were collected and submitted to the ISU VDL for SV-A testing.

Biosecurity and disease entry evaluation

A biosecurity and disease entry evaluation was conducted on 31 August 2015. The evaluation consisted of a standardized data collection and reporting outbreak assessment tool used in the United States hog industry for the investigation of PRRSV outbreaks. As the epidemiology of SV-A is poorly understood, the evaluation served as a hypothesis-generating tool to identify areas of potential entry and to create biosecurity recommendations to prevent entry of novel pathogens. The outbreak assessment tool was based on a comprehensive list of events that potentially led to the introduction of the virus into the breeding herd. The form included background information on the site, a description of the current outbreak and an evaluation of risks for how the pathogen entered the herd.

Results and Discussion

Herd evaluation and clinical observations

Of all sows in the farrowing house (n = 37) during the site visit on 31 August 2015, 72% had evidence of vesicular lesions on their snouts and 51% had hoof lesions. Of the sows with hoof lesions, 38% had partial separation of the hoof from the foot at the level of the coronary band and 92% had more than one foot affected (Fig. 1). Sows were agitated and reluctant to rise or expose udder and nipples to piglets. Of affected sows, 24% were anorexic. Mortality in piglets <7 days of age between 21 August and 28 August 2015 was 29% (n = 10 litters). Of litters born on 29 August 2015 and after, weekly PWM returned to baseline (approximately 15%). The increase in PWM during the initial week of the outbreak appeared to be transient and about 1 week in duration. About 50% of litters born between 24 and 30 August 2015 had piglets with mild diarrhoea and poor body condition. Temperatures of piglets and sows were not collected.

There were 20 sows housed in stalls in the gestation barn. Of these animals, 40% were lame and 60% had vesicular

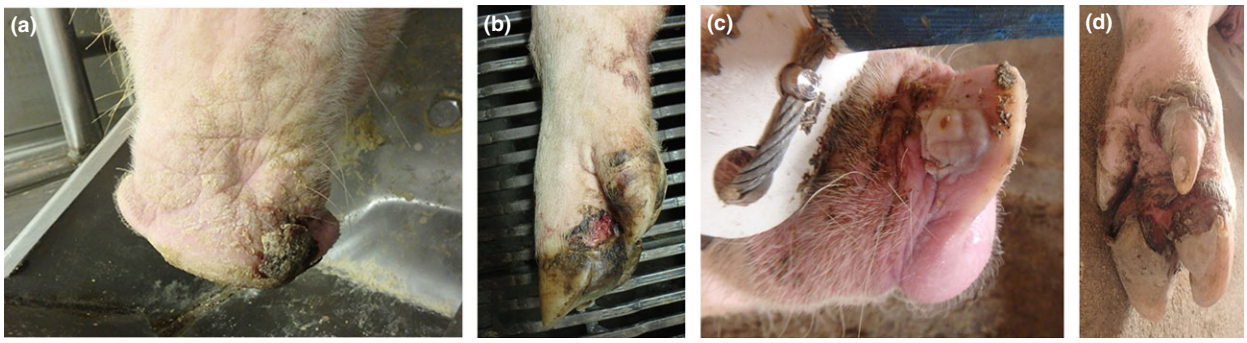

Fig. 1. Vesicular lesions on snout and feet of nursing sows (a and b) and gestating sows (c and d) on 31 August 2015.

lesions on the snout (Fig. 1). Gestating animals housed in pens could not be thoroughly evaluated for hoof lesions, but 40% demonstrated lameness; and 20% had snout vesicles. About 50% of breeding and teaser boars ($n = 6$) were also affected with lameness and vesicular lesions. Lameness or vesicular lesions were not observed in animals housed in the nursery or grower and finisher barns on the date of site evaluation. Rectal temperatures were not collected in any groups examined.

By the 11 September 2015 visit, all nasal vesicular lesions on sows observed on 31 August 2015 were no longer visible. Hoof lesions of these same sows were also healing well.

Diagnostics

Eight live piglets and one dead piglet from eight litters that had experienced wasting, a lack of vigour and sudden death were submitted to the ISU VDL for post-mortem examination and diagnostic testing by the owner on 25 August 2015. External examination was generally unremarkable. Five pigs were noticeably dehydrated. Upon visual assessment, two piglets had reddened coronary bands and three had equivocal skin abrasions immediately proximal to the hoof.

All nine piglets were necropsied with no internal gross lesions apparent. Tissues from each pig were collected and samples were pooled into two groups based on hydration, body condition and presence or absence of milk in stomachs. Group A pigs ($n = 4$) were in good body condition, well hydrated and had suckled recently. Group B pigs ($n = 5$) were dehydrated with empty intestinal tracts. Brain, lung, liver, kidney, stomach, intestine and colon sections from a portion of piglets in each group were preserved in formalin.

Necropsies of group A piglets revealed creamy colon contents. For group A, variable (low to high) populations of *Clostridium perfringens* and smooth/mucoid colon type *Escherichia coli* were isolated from the small intestines and colons, with moderate populations of *Salmonella* serogroup C1 also isolated from the colons. One group B piglet had mild mesocolonic oedema with creamy colon contents, but no other gross lesions were detected for group B.

Histological examination did not reveal consistent presence of lesions. In Group B, mild segmental acute loss of apical enterocytes with squamous metaplasia (mild atrophic enteritis) was present in two of the 14 sections of small intestine examined. Sections of colon were unremarkable in all nine piglets. No gross or microscopic lesions of diagnostic significance were observed in either group. Histological examination did not implicate a role for common enteric disease agents, nor was there evidence for bacterial septicaemia.

Samples for both groups tested negative on RT-PCR assays for foot-and-mouth disease virus (FMDV), porcine delta coronavirus (PDCoV), porcine epidemic diarrhoea virus (PEDV), porcine rotavirus types A, B and C, transmissible gastroenteritis virus (TGEV) and PRRSV. Testing for porcine circovirus type 2 (PCV-2) was not performed. Histological examination of these piglets did not reveal lesions compatible with PCV-2. Clinical signs or diagnosis of PCV-2-associated disease have not been documented on this farm. The most recent testing for PCV-2 on this farm was completed in February 2015 in association with an abortion case, and foetal tissues were negative for PCV-2 on RT-PCR. SV-A was detected by rRT-PCR from serum, skin, faeces and coronary band lesions from pigs submitted on 25 August 2015 (Table 1). Virus isolation attempts for SV-A using ST cells and sequencing for the VP1 gene region were successful from the piglet tissue samples (Knowles et al., 2006; Yang et al., 2012).

Faecal swabs and serum samples collected from piglets and sows during the site visit on 31 August 2015 were also positive for SV-A by rRT-PCR (Table 2). Due to funding restraints for additional diagnostics, other specimens collected such as tonsil swabs, vesicular scrapings, vesicular fluid swabs and nasal swabs from sows and piglets were held frozen at -80°C for additional testing if needed and for SV-A assay development. For the five piglets submitted from this visit, watery to pasty intestinal content was

Table 1. Results from Senecavirus A (SV-A) real-time reverse-transcription polymerase chain reaction (rRT-PCR) testing on samples submitted 25 August 2015

Specimen	Description	Average rRT-PCR SV-A Ct values
Faeces	Group A	24.6
Faeces	Group B	20.1
Skin	Pooled sample of groups A and B	20.9
Coronary Band	Pooled sample of groups A and B	25.2
Serum	Group A	22.4
Serum	Group B	26.1

Nine affected piglets were submitted for testing and amalgamated into two groups (A and B) based on clinical presentation and pooled for subsequent testing. Pig 5 in group B was deceased prior to submission, and serum SV-A rRT-PCR was not performed for this animal. All samples tested were positive for SV-A on rRT-PCR. A cycle threshold (Ct) value of ≤40 is considered positive.

Table 2. Results from Senecavirus A (SV-A) real-time reverse-transcription polymerase chain reaction (rRT-PCR) testing on samples submitted 31 August 2015

Specimen	Sample description	No. positive	Range of Ct values
Faecal Swabs	10 piglets	10	19.4–36.3
Faecal Swabs	10 sows	10	25.9–35.8
Serum	10 piglets	7[a]	19.2–35.8
Serum	10 sows	5	27.4–37.2

Faecal swabs and serum were collected from 10 sows in affected farrowing rooms and one piglet from each of their litters, totalling 10 sows and 10 piglets sampled. A Ct value of ≤40 is considered positive.
[a]Sows of all three SV-A negative piglets were also SV-A negative on serum by PCR.

observed in all animals. The morphological diagnosis for all pigs was enteritis characterized by minimal lymphoplasmacytic infiltration with villus oedema and diffuse mucosal erosion. The tonsil, heart, lungs, spleen, liver, kidneys, lymph nodes and brain were all histologically unremarkable. *Streptococcus suis* was isolated from lung from two pigs in few numbers. Haemolytic *E. coli* was isolated from intestine from one pig. Genotyping on the *E. coli* was not performed. SV-A rRT-PCR on tonsils was positive for all five pigs and faeces from four of the five pigs were positive for SV-A RNA. Molecular testing (PCR) for FMDV, PDCoV, PEDV, TGEV, porcine rotavirus types A, B and C, PCV-2 and PRRSV was not performed on this group due to previous negative results from 25 August 2015 submission and funding limitations.

During the 11 September 2015 follow-up visit, semen and a deceased rat were submitted for additional testing. The gel fraction of the semen was negative (Ct>40) for SV-A by rRT-PCR, and the sperm-rich fraction was positive for SV-A with a Ct value of 34.4. Virus isolation and sequencing of the SV-A VP1 gene were attempted on the sperm-rich fraction, but both were unsuccessful (Knowles et al., 2006; Yang et al., 2012). Faeces, oral swabs, tissue homogenate and foot pad swabs from the rat were all PCR negative (Ct>40) for SV-A.

Regulatory considerations

The state veterinarian was notified about the clinical presentation and updated on the status of the case when vesicles were identified by the herd veterinarian. At the time of reporting, there had been several foreign animal disease investigations conducted from cases of acute lameness and vesicular disease among exhibition and commercial market weight pigs (Rademacher, 2015; Zhang et al., 2015). For these concurrent cases, rRT-PCR testing results for FMDV, swine vesicular disease virus, vesicular stomatitis virus and vesicle exanthema of swine virus performed at the Foreign Animal Disease Diagnostic Laboratory (FADDL) at Plum Island were all negative. In this case, the state veterinarian did not request a foreign animal disease investigation. Therefore, samples were not sent to the FADDL for additional vesicular disease testing. Samples were tested negative for FMDV by rRT-PCR performed at ISU VDL, one of the National Animal Health Laboratory Network laboratories approved for presumptive FMDV PCR testing by USDA.

Biosecurity and disease entry evaluation

The events hypothesized to be high risks for the introduction of a novel pathogen to the farm were farm employee or repair personnel movements and the access of domestic animals and rodents to swine areas. Farm-specific clothing was worn by farm employees, but employees did not shower in or out, use a bench entry system or observe a line of separation. From 3 to 12 August 2015, repairs to the feed mill were made by service personnel with unknown previous swine contact. The repair personnel did not wear farm-specific clothing or boot covers, they entered the swine barn office, brought outside food into the farm site and shared equipment with the farm.

Although the extent of transmission of SV-A from terrestrial animals to swine is unknown, rodents, domestic cats and a dog had access to buildings housing swine, which was considered as a potential route of SV-A introduction. The farm is in close proximity (0.25 miles) to a sheep farm. There is one commercial site for growing hogs <2 miles from the site. Within 5 miles of the farm site, there is one sale barn for cattle, sheep and hogs and two exhibition pig sites.

Implications

Senecavirus A has been implicated in vesicular diseases among swine in North America and transient neonatal

death in Brazil; however, there has not been a clear, reproducible association between SV-A and piglet disease (Pasma et al., 2008; Yang et al., 2012; Vannucci et al., 2015). This is the first case report describing SV-A isolated from neonatal pigs where the clinical course of disease consisted of severe neonatal mortality of high morbidity among neonatal piglets, followed by vesicular lesions in the snouts and coronary bands of adult sows and boars in the United States.

Previous reports have described the detection of SV-A from cases of acute lameness, sudden neonatal death and vesicular disease in North and South America (Amass et al., 2004; Pasma et al., 2008; Vannucci et al., 2015). In Brazil, epidemic transient neonatal losses, a clinical syndrome in piglets similar to the case presented here, resulted in necropsied piglets with no specific gross or histopathologic lesions (Vannucci et al., 2015). Vannucci (2015) was able to demonstrate SV-A within the spleen, liver and vesicles of affected piglets using *in situ* hybridization. Furthermore, another report from Brazil also described the association of abundant quantity of SV-A RNA and vesicular lesions in pigs (Leme et al., 2015). From our case and the Brazilian cases, other causes of neonatal mortality were examined and ruled out. Further research is needed to understand whether SV-A is a primary cause of these conditions. Although virus has been identified in neonatal tissues of affected pigs, lesions are generally absent and Koch's postulates for neonatal mortality caused by SV-A have not been satisfied (Vannucci, 2015).

There were several limitations to the case investigation that centred on the collection of additional diagnostic specimens and the rapid response to clinical signs. Specifically, colostrum and milk were not sampled. It would be of value to compare SV-A viraemia in affected and non-affected piglets and sows to generate evidence for the association between the SV-A and neonatal mortality. Without a clear case definition and a small number of farrowings during the outbreak, it was challenging to select appropriate samples with significant sample size for a meaningful comparison. Lastly, the collection of samples in close proximity to the start of the outbreak would have provided better sample specimens and additional information to better characterize the outbreak in real time.

The presentation described here was atypical for cases of SV-A occurring in the United States during 2015, as clinical signs in the neonatal piglets preceded vesicular signs in adult animals by several days and growing stock remained unaffected. There is a need of further studies to clarify the role of SV-A on the development of vesicular disease and/or epidemic transient neonatal mortality in pigs. Animal caretakers and veterinarians must remain vigilant for vesicular foreign animal diseases and report clinical signs and lesions to the designated state animal health authority for diagnostic testing and further investigation. It is critical to rule out FMDV as the cause of disease as soon as possible.

Acknowledgements

The authors would like to acknowledge the operation owners and employees for their participation in the herd evaluation and collection of samples, fourth-year veterinary students Scott Radke, Christine Mainquist-Whigham, Colton Duxbury, Quinn Robinson for their assistance in sample collection, and Kristin Skoland and Tiffany Magstadt for the preparation of sample supplies. Resources for diagnostic testing were partially supplied by the Swine Health Information Center, Dr. Kyoung-Jin Yoon, Dr. Chris Rademacher, the Swine Medicine Education Center and the ISU VDL. The outbreak assessment data collection and reporting templates were supplied by the Iowa Pork Producers Association GS #122613.

References

Amass, S. F., J. L. Schneider, C. A. Miller, S. A. Shawky, G. W. Stevenson, and M. E. Woodruff, 2004: Idiopathic vesicular disease in a swine herd in Indiana. *J. Swine Health Prod.* 12, 192–196.

Hales, L. M., N. J. Knowles, P. S. Reddy, L. Xu, C. Hay, and P. L. Hallenbeck, 2008: Complete genome sequence analysis of Seneca Valley virus-001, a novel oncolytic picornavirus. *J. Gen. Virol.* 89, 1265–1275.

Knowles, N. J., L. M. Hales, B. H. Jones, J. G. Landgraf, J. A. House, and K. L. Skele, 2006: Epidemiology of Seneca Valley virus: identification and characterization of isolates from pigs in the United States. The Northern Lights EUROPIC 2006 - 14th Meeting of the European Study Group on the Molecular Biology of Picornaviruses. Saariselka, Inari, Finland. Abstract G2.

Leme, R. A., E. Zotti, B. K. Alcântara, M. V. Oliveira, L. A. Freitas, A. F. Alfieri, and A. A. Alfieri, 2015: Senecavirus A: An Emerging Vesicular Infection in Brazilian Pig Herds. *Transbound. Emerg. Dis.* 62, 603–611.

Pasma, T., S. Davidson, and S. L. Shaw, 2008: Idiopathic vesicular disease in swine in Manitoba. *Can. Vet. J.* 49, 84–85.

Rademacher, C., 2015: Case reports from Senecavirus A/Seneca Valley Virus – field experiences in Iowa. Allen D. Leman Swine Conference. University of Minnesota. Available at https://drive.google.com/file/d/0BzGsnfsQ28heLUZiN2Zv-SURZUVE/view (accessed October 19, 2015).

Reddy, P. S., K. D. Burroughs, L. M. Hales, S. Ganesh, B. H. Jones, N. Idamakanti, C. Hay, S. S. Li, K. L. Skele, A. J. Vasko, J. Yang, D. N. Watkins, C. M. Rudin, and P. L. Hallenbeck, 2007: Seneca Valley virus, a systemically deliverable oncolytic picornavirus, and the treatment of neuroendocrine cancers. *J. Natl Cancer Inst.* 99, 1623–1633.

Vannucci, F. A., 2015. Lessons learned from investigation of vesicular disease in swine – Rapid response and diagnosis

leading to a potential viral etiology and associated syndromes. Allen D. Leman Swine Conference. University of Minnesota. Available at http://traffic.libsyn.com/animalcast/150921_Leman_VesiDis_Vannucci.mp4 (accessed March 9, 2016).

Vannucci, F. A., D. C. Linhares, D. E. Barcellos, H. C. Lam, J. Collins, and D. Marthaler, 2015: Identification and complete genome of Seneca Valley Virus in vesicular fluid and sera of pigs affected with idiopathic vesicular disease, Brazil. *Transbound. Emerg. Dis.* 62, 589–593.

Yang, M., R. van Bruggen, and W. Xu, 2012: Generation and diagnostic application of monoclonal antibodies against Seneca Valley virus. *J. Vet. Diagn. Invest.* 24, 42–50.

Zhang, J., P. Piñeyro, Q. Chen, Y. Zheng, G. Li, C. Rademacher, R. Derscheid, B. Guo, K.-J. Yoon, D. Madson, P. Gauger, K. Schwartz, K. Harmon, D. Linhares, and R. Main, 2015: Full-length genome sequences of Senecavirus A from recent idiopathic vesicular disease outbreaks in U.S. swine. *Genome Announc.* 3, e01270–15.