Senecavirus A: Frequently asked questions

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Summary

Senecavirus A (SVA) has been demonstrated to be a causative agent for vesicular disease in swine. It is clinically indistinguishable from other agents that cause vesicular disease such as foot-and-mouth disease virus (FMDV), which is a reportable foreign animal disease (FAD). Thus, an investigation is initiated to rule out FMDV every time a vesicle is observed. Senecavirus A has now been reported across the Americas and Asia, and it appears the ecology of this virus has changed from sporadic infections to an endemic disease in some areas. In addition to vesicular disease, there have also been reports of increased neonatal mortality on affected sow farms. Knowledge about the pathogenesis of SVA in swine can provide many benefits to the swine industry. Understanding how long the virus can be detected in various sample types after infection can aide in choosing the correct samples to collect for diagnosis. In addition, the duration of virus shedding can help determine measures to control virus spread between animals. Prevention of SVA infection and disease with an efficacious vaccine could improve swine welfare, minimize SVA transmission, and reduce the burden of FAD investigations.

Resumen - Senecavirus A: Preguntas frecuentes

Se ha demostrado que el Senecavirus A (SVA) es un agente causal de enfermedad vesicular en cerdos. Es clínicamente indistinguible de otros agentes que causan enfermedades vesiculares como el virus de la fiebre aftosa (FMDV), que es una enfermedad exótica (FAD) de los animales y de declaración obligatoria. Por lo tanto, cada vez que se observa una vesícula, se inicia una investigación para descartar la presencia del FMDV. Actualmente se ha reportado la presencia del Senecavirus A en las Américas y Asia, además parecería que la ecología de este virus ha cambiado de infecciones esporádicas a una enfermedad endémica en algunas áreas. Además de la enfermedad vesicular, también se ha reportado un aumento de la mortalidad neonatal en las granjas de cerdas afectadas. El conocimiento sobre la patogénesis del SVA en cerdos puede proporcionar muchos beneficios a la industria porcina. Entender durante cuánto tiempo se puede detectar el virus en varios tipos de muestras después de la infección puede ayudar a elegir las muestras correctas a colectar para su diagnóstico. Además, la duración de la diseminación del virus puede ayudar a determinar las medidas para controlar la propagación del virus entre los animales. La prevención de la infección por SVA y la enfermedad mediante una vacuna eficaz podría mejorar el bienestar de los cerdos, minimizar la transmisión del SVA y reducir la carga de las investigaciones de FAD.

Résumé - Sénécavirus A: Foire aux questions

Le sénécavirus A (SVA) s'est avéré être un agent causal de maladie vésiculeuse du porc. Il est cliniquement impossible de le distinguer des autres agents responsables de maladie vésiculeuse, comme le virus de la fièvre aphteuse (FMDV), qui est une maladie animale exotique à déclaration obligatoire (FAD). Ainsi, une enquête est initiée pour écarter la fièvre aphteuse à chaque fois qu'une vésicule est observée. Le SVA a maintenant été signalé dans les Amériques et en Asie, et il semble que l'écologie de ce virus soit passée d'infections sporadiques à une maladie endémique dans certaines régions. En plus de maladie vésiculeuse, on a également signalé une augmentation de la mortalité néonatale dans les élevages de truies touchés. La connaissance de la pathogenèse de SVA chez le porc peut apporter de nombreux avantages à l'industrie porcine. Comprendre combien de temps le virus peut être détecté dans divers types d'échantillons après l'infection peut aider à choisir les bons échantillons à prélever pour le diagnostic. De plus, la durée de l'excrétion du virus peut aider à déterminer des mesures pour limiter la propagation du virus entre les animaux. La prévention de l'infection et de la maladie causées par SVA avec un vaccin efficace pourrait améliorer le bien-être des porcs, minimiser la transmission de SVA et réduire le fardeau des enquêtes sur les FAD.

Senecavirus A (SVA) is the only member of the genus *Senecavirus* in the family Picornaviridae.[1] The virus was first discovered in 2002 at a laboratory in Maryland as a cell culture contaminant in PER.C6 cells and was named Seneca Valley virus-001 (SVV-001).[1] It was speculated the contamination could have been introduced by either porcine trypsin or fetal bovine serum, both commonly used in cell culture.[2] The National Veterinary Services Laboratory isolated twelve picornavirus-like viruses

between 1988 and 2005 from swine exhibiting a variety of clinical signs and from multiple states across the United States.[3] Sequencing highlighted the close relationship of these isolates with SVV-001, and neutralizing antibodies were found in swine serum samples supporting swine as a natural host. Two of these historical isolates were used to inoculate pigs, but they did not develop any specific clinical disease.[3]

What clinical signs are observed during SVA infection?

Prior to 2014, SVA had only been detected in North America, and in a few cases, detection of virus was associated with an idiopathic vesicular disease in mature swine. In one report, market weight pigs being transported from Canada to the United States in 2007 arrived with vesicular lesions on the snouts and coronary bands.[4] Samples from these animals tested negative for the top differentials for swine vesicular disease: foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular stomatitis, and vesicular exanthema of swine; but, these animals did test polymerase chain reaction (PCR) positive for SVA.[4] Subsequently, in 2011 a boar from Indiana with vesicular lesions also tested PCR positive for SVA.[5] Due to evidence that SVA infected swine, a competitive enzyme-linked immunosorbent assay (ELISA) test was developed using serum generated from experimentally inoculated pigs.[6] Inoculated pigs did not develop clinical disease, though they did generate an antibody response. Thus, experimental infection with SVA failed to reproduce any consistent clinical disease, but evidence from field cases supported an association of SVA infection with vesicular disease in swine.

Beginning in late 2014, reports of vesicular lesions in swine along with an increase in neonatal mortality observed in piglets less than a week of age were spreading across the swine producing regions of Brazil.[7] The mortality observed in piglets was given the name epidemic transient neonatal losses (ETNL), and piglets displayed inconsistent clinical signs prior to death including lethargy, wasting, neurologic signs, and diarrhea.[7] Samples collected from these cases tested PCR and virus isolation (VI) positive for SVA.[8] Not only were these the first reports of SVA infection outside of North America, they also described

a different character of the field infections. Instead of sporadic, limited infections in a swine herd, the Brazilian reports describe an epidemic wave of vesicular disease in sows and ETNL in piglets through a swine dense region. In summer 2015, cases of vesicular disease in finishing pigs and sows with an increase in neonatal mortality were observed in US swine. Similar to field cases in Brazil, SVA was detected in the affected animals and genetic analysis found a 97.7% to 98.0% nucleotide identity to the isolates from Brazil.[9-11] Using 2015 SVA isolates from the United States, research groups were able to experimentally reproduce vesicular disease in 3-, 9-, and 15-week-old pigs,[12-14] confirming that SVA was a causative agent for vesicular disease in swine.

Since multiple groups were able to experimentally reproduce disease with the 2015 US SVA isolates, questions remain as to why previous attempts with historical isolates were unsuccessful. In retrospect, some previous animal inoculation reports provided limited information about methods including number of pigs inoculated, age, etc, so it is difficult to make direct comparisons to recent animal inoculations. Experimental studies with US isolates suggest lesions may be more difficult to see on younger animals or they may not develop at all, thus there may be age-related differences to expression of clinical disease. To date, all pigs experimentally inoculated with what is believed to be an infectious dose of SVA are susceptible to infection, but not all inoculated animals develop clinical disease leading to speculation that an individual pig may harbor different resistance/susceptibility traits. Beyond questions about the susceptibility of the host, there are questions about potential differences in pathogenicity of viruses or the requirement of a novel cofactor to explain possible differences in disease expression.

Although the first SVA cases reported in the Midwest involved pigs from county fairs and late finishing pigs with vesicular lesions on the coronary bands and snout, the virus was also quickly identified at sow farms reporting increases of neonatal mortality ranging from 30% to 70% along with a diverse range of adult animals exhibiting vesicular disease.[15] One study reported that 2 of 6 SVA-affected breeding herds did not report vesicular lesions in sows.[16] In Brazil, similar findings were reported consisting of neonatal mortality ranging from 5% to 60% and mixed reports on the

number of affected farms that also observed vesicular lesions on the sows.[17] A study comparing clinically affected sows to nonclinically affected sows on a farm experiencing an SVA outbreak demonstrated similar PCR-positive samples and antibody responses between both groups.[18] Therefore reiterating that not all animals infected with the virus develop vesicular disease and infected farms may be under reported, thus contributing to the spread of SVA.

What is the frequency of SVA detection?

Shortly after the 2015 outbreak began, 441 diagnostic cases including oral fluid samples from the United States tested via PCR had approximately 1% SVA prevalence.[19] During 2017, 444 diagnostic lab submission samples showed a 5.4% positive rate.[20] In addition, SVA antibodies in US swine were measured from samples collected in 2016 to better understand herd-level seroprevalence in both growing pigs and sows, and in the samples tested they estimated seroprevalence of 12.2% in growing pigs and 34% in sows.[21] In Brazil, serum samples prior to the 2014-2015 outbreak were negative for SVA antibodies while 34.6% of post-outbreak samples were positive, supporting that SVA had not been circulating in Brazil prior to 2014 and that seroprevalence was similar to US sow farms.[22] These levels of seroprevalence could also be suggestive of infections that went undetected due to missed clinical signs or lack of clinical signs.

How is SVA transmitted?

Epidemiologic investigations assigned employee entry, carcass disposal, and cull sow removal as high-risk events for SVA introduction to a farm, but also mentioned rodents, feed delivery, and semen entry as high risk.[16] Live virus has been isolated from environmental samples, mouse feces, and mouse small intestine from an affected farm; and virus was also detected via PCR in flies collected from both affected and unaffected farms, thus providing evidence that these pests may play a role in the spread of SVA between farms.[23] Recently, feed has been suspected as a vector to transport virus between countries.[24,25] Senecavirus A remained infectious in many feed ingredients tested in a simulated trans-Pacific Ocean journey and was shown to be the most stable of all the viruses studied.[26] Senecavirus A has been

detected in feed ingredients and complete feed samples collected from two feed mills in Brazil, but further research is needed to determine the risk of transmission through feed.[27] In addition, the daily trafficking of animals to slaughter plants provides abundant opportunity for virus spread between slaughter plants and trucks and back to farms or collection points. Trucks have been shown to play a role in the spread of viruses as demonstrated by studies with porcine epidemic diarrhea virus.[28,29] Recently, risk factors reported for SVA-positive pigs arriving at a slaughter plant included suppliers that raised pigs indoors and suppliers with pigs originating from multiple sites.[30]

Semen is a known risk for transmitting classical swine fever virus, porcine reproductive and respiratory syndrome virus (PRRSV), and pseudorabies (PRV) virus,[31] and with the detection of SVA PCR-positive semen, there is the potential for viral transmission during breeding.[11] Little objective data has been collected regarding cull sow movements; but one study found a significant number of sows entered multiple collection points prior to reaching a slaughter facility and traveled on average over 240 km from their site of origin, making the cull sow network a likely area of pathogen transmission.[32] Environmental samples from an assembly yard in Canada have tested positive for SVA.[33] In addition, samples from collection points in North Carolina from animals headed to slaughter tested PCR positive suggesting that SVA circulates in secondary and cull sow markets and most likely contributes to the spread of SVA.[19]

Pig-to-pig transmission has been observed both in the field and experimentally. Piglets weaned from an SVA-negative sow farm comingled with piglets from an SVA-positive sow farm tested positive for SVA in serum suggesting SVA spread among pigs during comingling, which is a common practice in the swine industry.[34] In experimental studies, sows that farrowed around 45 days after a challenge with SVA were still able to transmit virus to their piglets.[35] Unpublished research from our laboratory has demonstrated transmission of SVA to naïve contact sows from primary inoculated sows on 7 and 14 days post inoculation (dpi), but not on 21 or 28 dpi, so movement of infected animals can also play a role in SVA transmission. Live virus has been isolated from oral/nasal secretions and feces; therefore, fecal-oral transmission is likely an important route of transmission in addition to direct contact.[14]

What countries have reported SVA infection in swine?

Since the 2015 outbreaks reported in Brazil and the United States, vesicular disease cases due to SVA have been found across the globe including China,[36] Canada,[33] Colombia,[37] Thailand,[38] and Vietnam.[39] In March 2015, SVA was discovered and isolated in China with farms reporting vesicular lesions in sows and acute death in neonates.[40] In October 2015, sows transported to the United States from Canada had vesicular lesions on arrival which initiated a foreign animal disease (FAD) investigation. Tracebacks to the herd of origin detected SVA that was genetically similar to isolates found in China.[33] In February 2016, a breeding farm in Colombia broke with vesicular disease, and phylogenetic analysis of the SVA isolate clustered the Colombian strain with contemporary isolates from the United States (98.5%-98.9% nucleotide identity) rather than strains from Brazil (97.7%).[37] Senecavirus A was first detected in Thailand in October 2016 with lesions in market-weight pigs. At a genomic level, this virus was most closely related (98.2%) to the first Canadian strain from 2011.[38] Saeng-Chuto et al[41] suggested the SVA introduction to Thailand may not have been recent, but that the virus had evolved in the Thai swine population and remained undetected due to the presence of other pathogens that cause similar clinical disease, like FMDV. Finally, in January 2018, a group of pigs from Vietnam diagnosed with FMDV also tested positive for SVA, and the sequence shared high homology with isolates collected from China in 2015 and 2016.[39] This case and others highlight the difficulties faced by countries with FMDV and SVA cocirculating in swine herds to understand virus spread and mount control responses to each virus.

How genetically similar are SVA isolates?

Brazilian isolates appear to have originated from a common source, since they are genetically similar and group together in a clade separate from most US isolates from the same time period.[42] Surprisingly, early SVA isolates from China (2015-2016) tended to cluster together near Canadian and Brazilian isolates, while isolates from 2016-2017 began to cluster within the 2015 US isolates.[43-48]

Of note, one report commented that earlier isolates more closely related to Brazilian isolates caused acute death in neonates, while the more recent strains clustering with the US isolates did not cause mortality in piglets.[49] Clusters of Chinese isolates branch throughout SVA phylogenetic trees with little relationship to region or year of isolation and could imply multiple introductions into China or undetected circulation and adaptation in Chinese swine herds.[50,51] The genetic distance between the US and Brazilian isolates was reported to be 2.71%, while the distance between the US and China isolates was 2.48%, and 2.8% between Brazilian and Chinese isolates.[52] The overall genetic divergence of contemporary isolates was 2.8%, but the genetic divergence between contemporary isolates (2011-2017) and historical isolates (1988-2010) was 6.32%.[52] Another wave of outbreaks was reported in Brazil during the second half of 2018 in many of the same states that experienced outbreaks in 2015. Although the clinical disease presentation seemed more severe, phylogenetic analysis suggested the 2018 strains were not significantly different from those strains sequenced in 2015-2016 in Brazil.[53]

Is there evidence of recombination in swine?

There have been multiple reports of recombination events with Chinese SVA strains. A few events involving Chinese isolates from 2016-2019 showed parental strains from 2015 US isolates, SVV-001, a 2016 isolate from Colombia, and other Chinese isolates.[54-58] Thus, there is evidence of SVA recombination in China dating back to at least 2016. These events have been found to occur across the SVA genome.[57] Senecavirus A's RNA-dependent RNA polymerase has been shown to play a central role in SVA replicative recombination, and mutation rate was linked to recombination rate.[59] Though more research is needed in this area, evidence of recombination events has been reported in other picornaviruses, including FMDV, and can play a key role in virus evolution.[60]

How long are vesicular lesions observed?

Vesicular lesions in swine can be found on the coronary bands, intradigital space, snout, lips, and tongue.[61] Lesions can begin with erythematous areas or blanched areas of the skin progressing to vesicles with varying levels of fluid

that rupture leaving an erosion on the skin that crusts over and resolves.[14] Histologically, areas of separation between the dermis and epidermis with clefts are noted containing edema, fibrin, necrotic debris, and inflammatory infiltrates (neutrophils, lymphocytes, and plasma cells).[14,62] Development of vesicular lesions on the coronary bands have been observed in as little as 48 hours in market-weight animals, but most animals develop vesicular lesions 3 to 6 days after experimental challenge and heal within 7 to 14 days.[12-14,63,64] Snout lesion development has been described as delayed compared to the appearance of coronary band lesions and heals more quickly.[35,63] In addition, some studies have reported seeing fewer snout lesions compared to coronary band lesions.[62,65] Other clinical signs that have been reported intermittently in pigs inoculated with SVA include fever, lameness, lethargy, and decreased feed intake.[49,66]

Although most experimental infection studies with contemporary SVA strains have resulted in most animals developing vesicular lesions, field reports have described varying levels of incidence.[8,11,16,34] It is not understood why some animals develop vesicular lesions and others do not.[67] Exposure dose may play a role considering most experimental studies with swine have used inoculum doses between 10^7 and 10^8 median tissue culture infectious dose/mL, which may be higher than exposure levels in the field. Experiments with FMDV in swine have shown altered infection dynamics and a shorter time to clinical signs with higher doses of inoculum.[68,69] There has also been speculation surrounding age dependency of lesion development. One study, using an SVA isolate from China, inoculated pigs around 1, 2, and 3 months of age. The oldest pigs were the only pigs to develop vesicles on their coronary bands and snout while the two groups of younger pigs did not develop any visible lesions.[65] Differences in lesion development could also be affected by density of the SVA receptor, anthrax toxin receptor 1, on susceptible cells in the epithelium of the coronary band and snout, which could be dependent on age or genetics of the pig.[70]

How long do pigs replicate and shed SVA?

Viremia after experimental challenge lasts between 1 and 10 dpi with peak levels around 2 to 4 dpi.[12,71,72] Live virus has been isolated from serum on 2 to 3 dpi, but not later in infection.[61,72] Interestingly, it has been noted in recent experimental studies that not all challenged animals develop a viremia.[64] Oral, nasal, and rectal swabs typically test PCR positive from 1 to 21 dpi, but there are sporadic positive samples detected at an additional week or more with oral and nasal swabs often testing PCR positive longer than rectal swabs.[35,62] Virus isolation performed on swab samples was successful most reliably during the first week after inoculation, which coincides with peak RNA levels measured by PCR; although much less frequent, VI-positive oral and fecal swabs have been detected up to 21 dpi.[14,61,64]

Studies of SVA outbreaks in the field support observations from experimental studies. Shedding dynamics during a natural infection at a sow farm over 9 weeks post onset of clinical signs demonstrated vesicular lesions for approximately 2 weeks and viremia for approximately 1 week, but rectal and tonsil swabs from both piglets and sows were PCR positive for SVA at 6 weeks post outbreak.[34] Following an outbreak, a sow farm found PCR-positive rectal and tonsil swabs at least 6 weeks after the onset of clinical signs.[18] For diagnostics in the field with unknown infection status, swabs may be a more reliable sample than serum due to the greater longevity of SVA detection in swabs compared to serum. But, swabs of acute vesicular lesions are the best sample for the diagnosis of SVA due to the high levels of virus compared to other samples, however viral levels will likely decrease as lesions begin to heal.[63]

Does stress have an impact on SVA infection?

The role of stress and its effect on SVA pathogenesis and disease manifestation has been of interest following the first reports of SVA in the United States involving show pigs and finishing pigs being transported to market. Since experimental inoculation in the past had been unsuccessful, it was hypothesized that stress may be a required cofactor for clinical manifestation of SVA infection. This hypothesis was also supported by the fact that the virus had been found in healthy pigs without vesicular disease.[19,67] One early experimental inoculation with SVA treated half the pigs with an immunosuppressive regimen of dexamethasone prior to the challenge.

Both groups developed vesicular lesions and had similar infection dynamics, although a greater percentage of dexamethasone-treated pigs showed clinical signs approximately 24 hours earlier than those not treated.[62] Similarly, animals that were transported prior to challenge developed lesions slightly earlier than animals not transported prior to challenge, but viremia, shedding dynamics, and neutralizing antibody response were similar between both groups.[35] Thus, these studies support stress is not required for lesion development, but it may accelerate development.

Can SVA recrudesce in previously infected animals?

There had also been speculation of stress causing SVA to recrudesce or to renew active replication. Recrudescence has been reported to occur in other viruses that infect swine including the herpesvirus PRV.[73,74] A field study observed an increase in the percentage of piglets positive for SVA in serum at weaning almost 3 weeks after the virus had been cleared from the serum of most piglets.[34] An unconfirmed field report suggested that pigs without lesions present during marketing showed up to the slaughter plant approximately 12 hours later with lesions. Lesions were not observed on other animals from the farm of origin leading to speculation of recrudescence. Experimental work to test this theory challenged three groups of pigs with SVA and 46 days after infection applied a stressor event: transportation, dexamethasone treatment, or parturition. No lesions were observed after the stressor, but intermittent viremia and shedding was detected in all groups.[35] Of note, shedding detected by PCR was still reported in some animals at the stressor event from the initial SVA challenge.[35]

Can SVA cause persistent infection in swine?

The extended shedding seen in some animals infected with SVA could be attributed to persistence of the virus in tonsils. Live virus has been isolated from a tonsil 60 days after initial challenge, and *in situ* hybridization (ISH) localized the virus to both tonsillar epithelial cells as well as lymphoid tissues.[35] Double-stranded RNA (dsRNA) was detected by

immunofluorescence assay (IFA) in tonsils indicating a potential mechanism for persistence that has been shown for other viruses including PRRSV.[75,76] In addition, dsRNA is also a product of positive-sense RNA viral replication, so the dsRNA could also represent continued replication of SVA in the tonsillar tissue. Sows that farrowed approximately 46 days after initial exposure to SVA were able to transmit virus to their piglets supporting continued replication in animals long after the resolution of clinical signs.[35] Although piglets were found positive for SVA, the piglets did not demonstrate any clinical signs. Unpublished work from our group where neonates were experimentally challenged with SVA also did not result in any clinical signs, which further perpetuates the mystery surrounding ETNL in the field and the inability to reproduce that syndrome experimentally.

How does SVA infection impact neonates?

Piglets in Brazil have been reported to have ulcerative lesions on the snout, tongue, and coronary bands in SVA-affected farms; however, those lesions have not been reported frequently in piglets in the United States.[77,78] Brazil also had more reports of neurologic disease in neonates, which was supported by immunolabeling of SVA found in the choroid plexus of the brain and the surrounding endothelium cells of blood vessels of piglets that died on SVA-affected farms.[77] In addition, piglets submitted to a diagnostic lab in Brazil for ETNL had atrophic enteritis with positive immunolabeling in apical enterocytes as well as transmission electron microscopy evidence of viral particles similar in size and morphology to that of picornaviruses in the apical enterocytes.[79] Senecavirus A was also detected in urinary epithelium by immunohistochemistry (IHC) with ballooning degeneration of the transitional epithelium.[80] Histology and IHC demonstrated a multi-systemic infection of SVA in piglets; and quantification by PCR had demonstrated that the lymphoid organs had the highest levels of virus, which has also been observed in older swine after an experimental challenge.[62,81] Lesions and virus in the urinary and enteric tracts suggest that urine and feces could be a mode of horizontal transmission of SVA. Detection of SVA by IHC in tissues from 1- to 2-day-old piglets also suggests vertical transmission of the virus from sows.[80]

Are contemporary isolates more pathogenic than historical isolates?

Due to the inability of early studies to reproduce clinical disease with an experimental SVA challenge, we hypothesized that older isolates were less pathogenic than contemporary SVA isolates. Our work showed that both contemporary and historical isolates, including SVV-001, were able to cause vesicular disease in swine.[82] In contrast, another study reported that pigs challenged with SVV-001 did not develop vesicular lesions, while the group challenged with a 2015 isolate did develop clinical disease.[64] Both groups developed cross-neutralizing antibodies and cross-neutralizing T-cell responses suggesting conserved antigenic determinants, which was also supported by the cross-neutralizing titers in our study.[64,82] Another group compared the pathogenicity of two contemporary Chinese isolates (2016 vs 2017) and found one isolate to be more pathogenic in pigs than the other, with the 2016 isolate most closely related to Canadian isolates not causing vesicular disease in a group of finishing pigs.[71] Recent cell culture work with 5 different Chinese isolates located in different SVA phylogenetic clusters also showed small differences in viral growth kinetics in swine testicular cells.[83] Therefore, more research is necessary to correlate SVA genomic differences with pathogenic differences in SVA strains.

What is the humoral and cell-mediated immune response to infection?

Neutralizing antibody titers have been measured in pigs as early as 3 to 5 days after experimental infection, which has been correlated with VP2- and VP3-specific IgM responses.[12,14,63,72] This rise in neutralizing antibodies corresponds with the decline in viremia. Immunoglobulin G antibody response to infection follows IgM with titers beginning around 10 dpi.[14,62] Surface protein VP2-specific IgG antibodies were detected longer than VP1 and VP3.[72] Neutralizing antibodies have been found in animals up to 5 months after initial exposure, but further research must be performed to determine the protective titer. Critical for antibody production, CD4+ helper T cells were detected by 7 dpi, while CD8+ and CD4+ CD8+ T cells (effector/memory) increased after 10 dpi.[72] Aforementioned VP2-specific responses were highly suggestive that VP2 contains important B-cell and T-cell epitopes.[72]

What diagnostics are available for SVA?

An invaluable tool for virus detection and SVA diagnosis is PCR. Both SYBR Green and TaqMan-based real-time reverse transcriptase-PCR (qRT-PCR) assays have been developed with probes targeting different regions of the virus, including VP1 and 3D.[84-86] In addition, a nested-PCR assay has been developed to amplify a fragment of VP1, which was able to identify SVA RNA in samples considered negative by reverse transcriptase-PCR (RT-PCR).[87] A real-time reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed to provide a cheaper option for SVA detection.[88] The RT-LAMP procedure has also been combined with a lateral flow dipstick for rapid visualization of results.[89] Finally, an RNA RT-droplet digital PCR was also developed to allow quantification without the need for standard curves and is resistant to inhibitors present in different sample types.[90]

Not only is it important to be able to detect the virus in swine via PCR, it is equally important to be able to measure the antibody response to infection. As opposed to PCR, serology can provide information on SVA exposure over time. To identify SVA antibodies in swine, SVA VP1 and VP2 recombinant protein indirect ELISAs have been developed, which are more rapid and convenient for diagnostic labs versus assays that involve cell culture, like virus neutralization (VN) assays and indirect IFA.[18,91] One group found antibody responses to VP2 were higher than VP1 and VP3 and had higher binding affinity in the ELISA, which correlates with data that VP2-specific IgG antibodies were shown to last the longest in experimentally challenged pigs.[72,91] Indirect ELISAs can have high-cross reactivity, so a competitive ELISA has also been developed.[6,92] Although the scalability of the ELISA assay is favored by diagnostic labs, VN and IFA assays are excellent confirmatory assays with high sensitivity and specificity and often used in research settings.[14,92] Recently an enhanced green fluorescent protein tagged recombinant SVA has been developed to facilitate reading VN assays.[93]

Both ISH and IHC assays have been developed to detect SVA within tissues.[78,80] Immunohistochemistry assays detect

viral antigen and require antibodies against the virus of interest, which in the case of SVA can be difficult to obtain commercially. In contrast, for RNAScope (ISH), a probe is ordered to a target genomic region of interest for virus detection in tissues.[14,78] Although it is rarely used for clinical diagnosis, electron microscopy has also been used to identify particles with picornavirus morphology in animals infected with SVA.[79]

How can SVA be differentiated from other vesicular disease-causing viruses?

The ability to differentiate between viruses that cause vesicular disease in swine is important since they are clinically indistinguishable. This is especially significant for FMDV, since it is on the World Organization for Animal Health list of notifiable diseases.[94] To this end, a multiplex qRT-PCR assay was developed for quick differentiation of FMDV and SVA.[20] Multiplex assays are particularly vital for those countries that have multiple endemic viruses that cause vesicular disease in swine because the ability to track different viruses will be critical to understanding viral epidemiology to develop control and prevention plans. Of equal importance to differentiation is the speed at which the diagnosis can be made. In countries free of FMDV, there is a halt to swine movement when a vesicle is observed until FMDV has been ruled out. Pen-side testing allows quicker results and could contribute to faster continuity of animal movements. To this end, a field-deployable RT-insulated isothermal PCR (RT-iiPCR) has been developed that can detect SVA in the field.[95] Unfortunately, this test can diagnose SVA, but it does not provide information about FMDV status.

For countries with FMDV-negative status, identifying FMDV would have severe economic ramifications including production loss, trade restrictions, control measures, and the cost of regaining FMDV-free status.[96] Due to the significant consequences involved with an FMDV-positive diagnosis, testing for FMDV is highly regulated. In the United States, when a vesicle is observed in swine, a foreign animal disease investigation (FADI) is instigated. Trained personnel collect a set of standard samples in duplicate to be sent to both the Foreign Animal Disease Diagnostic Lab and a National Animal Health Laboratory Network lab to rule out FMDV. Therefore, even though pen-side diagnostic tests for FMDV have been produced (RT-iiPCR and lateral flow device), governments may be reluctant to approve these platforms due to ramifications of false-positive/negative results.[97-99] For example, a false-negative result could lead to the movement of positive animals and contribute to the spread of FMDV, which is considered one of the most highly contagious animal diseases.[100]

What disinfectants and inactivation techniques work against SVA?

Disinfectants have shown differing levels of success at inactivating SVA on different surfaces at various temperatures. In one study, bleach (sodium hypochlorite) at a 1:20 dilution was most effective at inactivating the virus, with a quaternary ammonium disinfectant demonstrating intermediate success depending on surface and temperature, and a phenolic disinfectant performing the worst.[101] Accelerated hydrogen peroxide at 1:20 for 10 minutes was also an effective disinfectant against SVA, as well as FMDV and SVDV.[102] Ultraviolet-C (254 nm wavelength) can also be used as an inactivation method, though it may be best suited as a redundant biosecurity measure because it was seen to be less effective with nonenveloped viruses and required greater than 3000 J/L for viral inactivation of SVA.[103]

Trypsin was suspected to be the source of contamination when SVA was discovered as a cell culture contaminant due to evidence of swine being the natural host for SVA and since porcine trypsin is used commonly in cell culture work. Some swine vaccines are grown in cell culture, thus raising concern for SVA contamination during the vaccine manufacturing process. Vaccine distribution nationally and around the globe could serve as a route for dissemination of SVA. Two lots of trypsin that had received 25 to 40 kGy of gamma-irradiation tested PCR positive for SVA and also VI positive indicating live virus.[104] Of note, after the trypsin samples received a second round of gamma-irradiation, SVA was inactivated. Thus, animal biologic manufacturers using porcine trypsin should add SVA to their exogenous agent testing to ensure that SVA is not inadvertently being spread through swine biologics.

What vaccines are available to protect swine against SVA infection?

Multiple vaccine platforms have been evaluated for efficacy against an SVA challenge. A whole-virus inactivated vaccine made from a Chinese SVA isolate mixed with an adjuvant given in one dose provided protection against a homologous challenge by preventing the development of clinical signs and viremia.[105] Similarly, unpublished work from our research group has shown the efficacy of a whole-virus inactivated vaccine (2015 US SVA isolate) in both weaned pigs and sows. In addition, piglets suckling immunized dams were protected against an SVA challenge. Also, a recombinant SVA strain used as a live attenuated vaccine given in a single dose induced a robust antibody response; and after a challenge with SVA, animals did not develop any clinical disease, had reduced viremia, and had reduced viral shedding compared to nonvaccinated animals.[106] Interestingly, an inactivated vaccine tested in the same study did not produce detectable neutralizing antibodies until after a second dose was given; and after the challenge, the inactivated SVA vaccine did not protect against the development of vesicular disease.[106]

Recently, a virus-like particles (VLP) vaccine for SVA has been tested in swine against an SVA challenge. A VLP vaccine consists of viral structural proteins that spontaneously self-assemble into particles antigenically indistinguishable from the native virus.[107] An advantage of VLP vaccines is they present viral antigens in a more authentic conformation compared to typical subunit vaccines with recombinant proteins.[108] Pigs vaccinated with SVA VLP and challenged with a 2017 Chinese isolate did not develop clinical disease or viremia. This study also showed similar efficacy of a one-dose inactivated virus vaccine. Having an effective commercial vaccine for SVA could reduce the occurrence of SVA-related vesicular disease, thus reducing the economic burden of FADIs in FMDV-free countries and viral load of SVA in endemic regions.

What are the next steps?

The change in SVA ecology from rare infections detected in the United States and Canada to small epidemics in Brazil, then the United States, and subsequently other countries around the

world, produced many questions about this virus. Since the fall of 2015, when SVA was demonstrated as a causal agent of vesicular disease in swine, several questions about the biology and pathogenesis of the virus have been addressed through research conducted in many laboratories and will continue to be addressed with future research. However, questions remain about why the SVA paradigm changed around 2015. Are the SVA epidemics reported around the world related? Have properties of the virus changed? What is the relationship between SVA and neonatal morbidity and mortality in the field?

As SVA spreads around the globe it will continue to present challenges due to its clinical similarity with FMDV. If SVA becomes endemic in FMDV-free regions, there is danger of the swine industry becoming complacent in reporting vesicular lesions by assuming these lesions are due to SVA. Improving knowledge through research about the epidemiology, viral evolution, and pathogenesis of SVA may help focus swine industry efforts directed at controlling the spread of SVA and future elimination efforts.

Acknowledgments

Conflict of interest

None reported.

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