

Stability of Senecavirus A in animal feed ingredients and infection following consumption of contaminated feed

Leonardo C. Caserta^{1,2} | Jessica C. G. Noll^{1,2} | Aaron Singrey¹ |
Megan C. Niederwerder³ | Scott Dee⁴  | Eric A. Nelson¹ | Diego G. Diel^{1,2}

¹ Department of Veterinary and Biomedical Sciences, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, South Dakota, USA

² Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA

³ Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

⁴ Pipestone Applied Research, Pipestone Veterinary Services, Pipestone, Minnesota, USA

Correspondence

Diego G. Diel, Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, 240 Farrier Road, A3-114, Ithaca, NY 14853, USA.
Email: dgdziel@cornell.edu

Funding information

Swine Health Information Center,
Grant/Award Numbers: 18-137, 18-211

Abstract

Animal feed and feed ingredients have recently been investigated as sources of pathogen introduction to farms and as a potential source of infection to animals post-consumption of contaminated feed. Survival of several viruses for a prolonged period has been demonstrated in feed. Here, we determined the rate of decay of Senecavirus A (SVA) in swine feed ingredients as a function of time and temperature and established half-life estimates for the virus. Select feed ingredients were spiked with a constant amount of SVA (10^5 median tissue culture infectious dose 50) and incubated at 4, 15 and 30°C for up to 91 days. Virus viability and the presence of viral RNA were assessed in samples collected over time. At the three different temperatures investigated, dried distillers' grains with solubles (DDGS) and soybean meal (SBM) provided the most stable matrices for SVA, resulting in half-lives of 25.6 and 9.8 days, respectively. At 30°C, SVA was completely inactivated in all feed ingredients and in the control sample, which did not contain a feed matrix. Although virus infectivity was lost, viral RNA remained stable and at consistent levels throughout the experimental period. Additionally, the ability of SVA to infect swine via ingestion of contaminated feed was investigated in 3-week-old, weaned pigs. Animals were provided complete feed spiked with three concentrations of SVA (10^5 , 10^6 and 10^7 per 200 g of feed) and allowed to naturally consume the contaminated feed. This procedure was repeated for three consecutive days. Infection of pigs through consumption of contaminated feed was confirmed by virus neutralization assay and the detection of SVA in serum, feces and in the tonsil of exposed animals by real-time reverse transcriptase PCR. Our findings demonstrate that feed matrices are able to extend the survival of SVA, protecting the virus from decay. Additionally, we demonstrated that consumption of contaminated feed can lead to productive SVA infection.

KEYWORDS

feed biosecurity, half-life, ingredients, Senecavirus A, SVA, swine diseases

1 | INTRODUCTION

The survival of viral pathogens in animal feed and feed ingredients and the risk of pathogen transmission through feed have received significant attention of the swine industry in recent years (Dee et al., 2016, 2018; Niederwerder and Rowland, 2017). Senecavirus A (SVA), a vesicular disease-causing agent of swine (Joshi, Fernandes et al., 2016; Montiel et al., 2016), is highly stable and demonstrated broad survivability across multiple feed ingredients (Dee et al., 2018). This can be attributed in part to the physicochemical properties of the virus. SVA is a non-enveloped, single-stranded, positive-sense RNA virus of the genus *Senecavirus*, family *Picornaviridae* that is resistant to broad pH variations (Venkataraman et al., 2008). Members of the *Picornaviridae* family, which also includes enterovirus A through J, rhinovirus-A, -B and -C, cardiovirus A and B and foot and mouth disease virus (FMDV) (Zell et al., 2017), are recognized for their stability in diverse environmental conditions. Since 2014, an increased number of outbreaks of vesicular disease caused by SVA have been reported worldwide, affecting swine in the United States (Guo et al., 2016; Joshi, Mohr et al., 2016) and other major swine-producing countries across the world, including Brazil (Leme et al., 2019a), China (Wu et al., 2017), Thailand (Saengchuto et al., 2018), Colombia (Sun et al., 2017) and Vietnam (Arzt et al., 2019). SVA causes lesions that are clinically indistinguishable from foot and mouth disease and other vesicular diseases of swine, with affected pigs presenting vesicles on the snout, oral mucosa and coronary bands, whereas clinical signs include lameness and lethargy (Chen et al., 2016; Joshi et al., 2016; Maggioli et al., 2018; Montiel et al., 2016; Leme et al., 2017).

The role of feed on transmission of SVA remains unknown. However, detection of SVA RNA in feed samples collected in the field in Brazil (Leme et al., 2019b) and the demonstrated stability of infectious virus in feed and feed ingredients for up to 37 days (Dee et al., 2018) suggest that contaminated feed could potentially serve as a fomite for introduction of SVA in susceptible swine populations. Feed was also implicated as a potential risk factor for the introduction and dissemination of porcine epidemic diarrhoea virus (PEDV) in commercial farms in the United States and of African swine fever (ASFV) in swine herds in Europe and Asia (Oļševskis et al., 2016; Pasick et al., 2014; Wen et al., 2019; Zhai et al., 2019). The potential risk of introduction of swine viruses posed by importation of feed ingredients from foreign countries has led to the investigation of the stability of other viruses in swine feed. Experimental data showed that contaminated feed can serve as a vehicle for PEDV and ASFV resulting in productive infection of pigs after consumption of contaminated feed (Dee et al., 2014; Niederwerder et al., 2019). Importantly, feed ingredients seem to provide a stable matrix that extends the half-lives of different swine viruses under moderate environmental conditions (~10°C) (Dee et al., 2018; Stoian et al., 2019). The effect of temperature on the survival of SVA in feed, however, has not yet been investigated.

Given that animal feed is stored and transported under variable environmental conditions, including different temperatures throughout different seasons of the year, here we sought to assess the effect of different temperatures on the viability of SVA in a select group of

feed ingredients. Virus inactivation kinetics were used to determine the half-life ($T_{1/2}$) of the virus as a function of time and temperature. Additionally, the infectivity of SVA following oral consumption of contaminated feed was investigated in pigs.

2 | MATERIALS AND METHODS

2.1 | Sample inoculation, incubation and processing

Five grams of gamma-irradiated soybean meal (SBM), dried distillers' grains with solubles (DDGS), lysine and vitamin D were placed in 50-ml sterile conical tubes. Each feed ingredient was spiked with 10^5 median tissue culture infectious dose 50 (TCID₅₀) of SVA strain SD15-26 in 100 µl of minimum essential medium (MEM) and mixed by vortexing for ~10 s. Three independent experiments were performed and, in each experiment, duplicate samples were inoculated, in a total of six samples per time point at each temperature. Controls consisted of virus inoculum placed in the plastic tube with no feed matrix (positive control). Samples were incubated at 4, 15 and 30°C and collected and processed according to the time points shown in Table 1. The content of each tube was resuspended in 15 ml of phosphate-buffered saline (PBS) and vortexed for 10 s. Samples were clarified by centrifugation at 5000 × g for 10 min at 4°C and supernatants were aliquoted and stored at -80°C for further analyses.

2.2 | Virus titrations

The amount of infectious virus in each sample and time point was determined by end-point dilutions in H1299 cells. Supernatants from the processed samples were subjected to 10-fold serial dilutions (10^{-1} to 10^{-6}) in RPMI 1640 medium supplemented with 10% foetal bovine serum. Each dilution was inoculated into four wells of a 96-well plate. Next, 100 µl of a H1299 cell suspension was added to each well. Plates were incubated at 37°C with 5% CO₂ for 48 h, and cells were fixed with 80% acetone for 20 min and stained with an anti-SVA VP2 FITC-conjugated monoclonal antibody (1:300 in PBS; SD214-188; kindly provided by Dr. Eric Nelson and Steve Lawson, South Dakota State University). After 1-h incubation at 37°C, cells were washed three times with PBS and evaluated under a fluorescence microscope.

Viral titres were calculated by the Spearman and Karber's method and expressed as TCID₅₀/ml. To determine the total amount of SVA present in each feed ingredient and time point of the experiment, the titres were normalized to the volume of PBS used to reconstitute the feed ingredient by multiplying the viral titre per millilitre by 15 (15 ml of PBS used to reconstitute 5 g of feed ingredients).

2.3 | Calculation of SVA inactivation time

Half-life ($T_{1/2}$) estimates and corresponding 95% confidence intervals (CI) were calculated by using the resultant slopes of simple linear

TABLE 1 Sampling schedule/timetable

| Days p.i. | 4°C | | | | 15°C | | | | 30°C | | | |
|-----------|-----|------|-----------|--------|------|------|-----------|--------|------|------|-----------|--------|
| | SBM | DDGS | Vitamin D | Lysine | SBM | DDGS | Vitamin D | Lysine | SBM | DDGS | Vitamin D | Lysine |
| 1 | x | x | x | x | x | x | x | x | x | x | x | x |
| 3 | - | - | - | - | - | - | x | x | x | x | x | x |
| 5 | - | - | - | - | - | - | x | x | x | x | x | x |
| 7 | x | x | x | x | x | x | x | x | x | x | x | x |
| 9 | - | - | - | - | - | - | x | x | x | x | x | x |
| 11 | - | - | - | - | - | - | x | x | x | x | x | x |
| 14 | x | x | x | x | x | x | x | x | x | x | x | x |
| 21 | x | x | x | x | x | x | x | x | x | x | x | x |
| 28 | x | x | x | x | x | x | | | | | | |
| 35 | x | x | x | x | x | x | | | | | | |
| 42 | x | x | x | x | | | | | | | | |
| 49 | x | x | | | | | | | | | | |
| 56 | x | x | | | | | | | | | | |
| 63 | x | x | | | | | | | | | | |
| 70 | x | x | | | | | | | | | | |
| 77 | x | x | | | | | | | | | | |
| 84 | x | x | | | | | | | | | | |
| 91 | x | x | | | | | | | | | | |

regressions of $\log_{10} Y$ (titres) on X (time) in the equation $-\log(2)/\text{slope}$, as previously described (Bryan et al., 1990). Linear regression models were applied for each feed ingredient at each temperature using the GraphPad Prism version 8 software (GraphPad Software Inc). Lower and upper confidence intervals were calculated using the equation $-\log(2)/\text{lower slope}$ or $-\log(2)/\text{upper slope}$. Complete results of linear models are provided in Table S1.

2.4 | SVA RNA detection by real-time reverse transcriptase PCR

RNA was purified from the supernatants of processed feed ingredients and controls using the Cador Pathogen 96 kit (Indical Biosciences) and a QIAcube HT automated nucleic acid extractor (Qiagen) following the manufacturer's instructions. Samples were tested for the presence of SVA RNA by real-time reverse transcriptase PCR (rRT-PCR) using the TaqMan Fast Virus 1-Step Master Mix kit (Applied Biosystems) with primers and probe targeting the SVA 3D polymerase gene: 5'-/56-FAM/CAGGAACAC/ZEN/TACTCGAGAAGCTGCAA/3IABkFQ/-3', 5'-GAAGCCATGCTCTCCTACTTC-3' and 5'-GGGTGCATCAATCTATCA-TATTCT TC-3' (Sharma et al., 2019). Results were expressed as genome copy numbers per millilitre, which were calculated using the relative quantitation method and based on a standard curve prepared with

serial dilutions a SVA virus stock with known infectious titer (Sharma et al., 2019).

2.5 | Experimental infection through SVA spiked feed

To assess the infectivity of SVA in pigs via oral consumption, we performed an animal experiment in which animals were fed SVA-spiked growth pig feed (Figure 1). For this, twelve 3-week-old piglets were randomly allocated into three groups as follows: group one (G1) received feed spiked with 10^5 TCID₅₀ of SVA SD15-26 per 50 g of feed ($n = 4$), group two (G2) received feed spiked with 10^6 TCID₅₀ of SVA SD15-26 per 50 g of feed ($n = 4$) and group three (G3) received feed spiked with 10^7 TCID₅₀ of SVA SD15-26 per 50 g of feed ($n = 4$). The animals were subjected to 7 days of acclimation prior to receiving contaminated feed. Each group was housed in separate pens distant from each other in a large BSL-2 room. Animals were always handled from the lower to the higher dose group. Animals were subjected to a 12-h feed withdrawal and then fed 200 g (~50 g per pig) of contaminated feed early in the morning for three consecutive days (day 1 through 3). After the animals ingested all the SVA-contaminated feed, they were provided noncontaminated feed for the rest of the day. Samples of serum and rectal swabs were collected at days 0, 3, 7 and 14 post-infection (dpi).

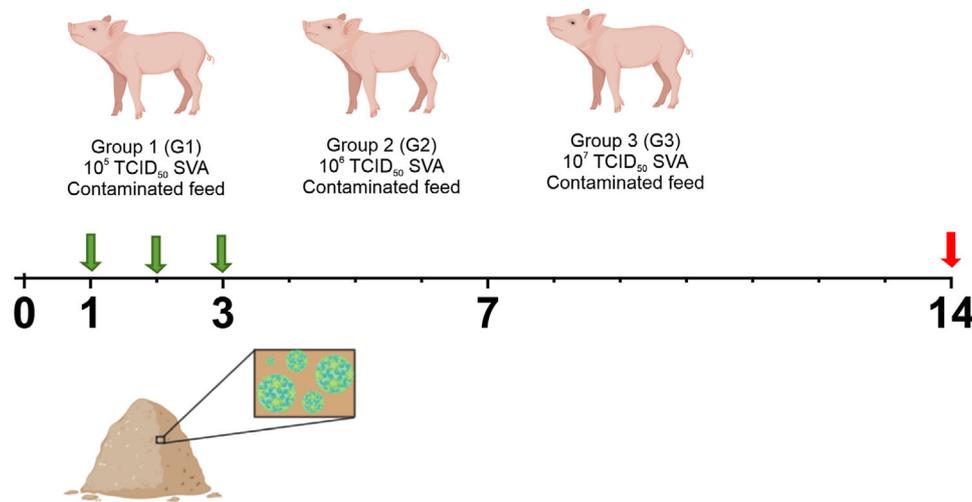


FIGURE 1 Experimental design for bioassay. Group one (G1) received feed spiked with 10^5 TCID₅₀ of SVA SD15-26 per 50 g of feed ($n = 4$), group two (G2) received feed spiked with 10^6 TCID₅₀ of SVA SD15-26 per 50 g of feed ($n = 4$) and group three (G3) received feed spiked with 10^7 TCID₅₀ of SVA per 50 g of feed ($n = 4$). Animals were offered SVA-contaminated feed for three consecutive days (day 1 through 3). Samples of serum and rectal swabs were collected at 0, 3, 7 and 14 days post-infection (dpi). At 14 dpi, the animals were euthanized, and the tonsils were collected for virological assessments

At 14 dpi, the animals were euthanized, and the tonsils were collected and stored at -80°C . All animal studies were reviewed and approved by South Dakota State University Institutional Animal Care and Use Committee (Committee approval no. 18-083A).

2.6 | Animal sample processing and testing

Serum and rectal swab samples were collected on 0, 3, 7 and 14 dpi, and the tonsil samples were collected at necropsy on 14 dpi. These samples were tested for the presence of SVA RNA using rRT-PCR. Nucleic acid was extracted from serum, rectal swabs and tonsils using the Cador[®] Pathogen 96 kit (cat no.54161) and the QIAcube HT (QIAGEN cat no. ID 9001793) automated extractor according to the manufacturer's instructions. The tonsils were first homogenized in MEM (1:10 [w/v] ratio), and 200 μl of the homogenate was used for nucleic acid extraction. For all extractions, a dilution of 10^4 TCID₅₀ of the SVA SD15-16 virus stock was prepared in plain MEM and used as positive control. A SVA negative serum sample was used as negative control.

The rRT-PCR reaction was performed using the commercial kit EZ-SVA RT-PCR (Tetracore Inc., catalogue number TC-90079-192). Each reaction was prepared by mixing 17.25 μl of master mix, 0.75 μl of enzyme and 7 μl of template RNA. The amplification/detection conditions for these rRT-PCR reactions were as follows: a reverse transcription step consisting of one cycle of 48°C for 15 min followed by one cycle of 95°C for two min; then one cycle of polymerase activation (95°C , 2 min) and 40 cycles of denaturation and annealing/extension (95°C , 5 s followed by 60°C , 40 s). A standard curve was generated with serial 10-fold dilutions of the SVA SD15-16 virus stock to determine the limit of detection of primers and probe. The dilution series were subjected to nucleic acid extraction and rRT-PCR amplification.

The detection limit of 1 TCID₅₀ equivalent per reaction (equivalent to a CT value of 39.31) was used as a cut-off value for the rRT-PCR. All samples with CT values higher than the cut-off were considered negative.

3 | RESULTS

3.1 | Viability of SVA in feed ingredients under different temperatures

The effect of temperature on SVA decay over time was assessed by measuring the viral titres recovered from each ingredient harvested at different time points. The dynamics of viral titres over time determined in SBM, DDGS, lysine and control are presented in Figure 2. SVA remained viable for prolonged times in SBM and DDGS across all three temperatures tested. Viability of the virus rapidly decreased in vitamin D and lysine (Figure 2).

When SVA-spiked feed ingredients were incubated at 4°C , viable SVA was recovered from DDGS and SBM until day 91 of incubation. DDGS and SBM presented a log₁₀ reduction of 1.08 and 2.91, respectively, indicating greater virus survival in DDGS. The higher stability was observed in DDGS at 4 and 15°C and for SBM at 30°C (Figure 2), suggesting that SBM provides a protective environment for the virus at higher temperatures in comparison with DDGS. At 30°C , SVA was inactivated by day 14 and 21 post-incubation in DDGS and SBM, respectively (Figure 2).

In contrast, the stability of SVA in lysine and vitamin D was greatly reduced when compared to DDGS and SBM. Incubation in vitamin D and lysine at 15 and 30°C resulted in inactivation of the virus within 1 day, whereas at 4°C the virus was stable until day 14 of incubation in lysine (Figure 2).

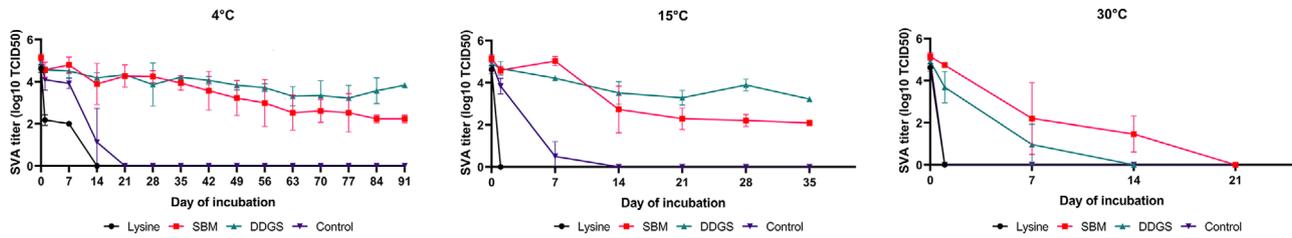


FIGURE 2 Stability of SVA kinetics in animal feed ingredients under different temperatures. Five grams of each feed ingredient were inoculated with 10^5 TCID₅₀ of SVA, incubated at 4, 15 or 30°C and processed for microtitration assays. SVA titres are expressed as log₁₀ median tissue culture infectious dose 50 (TCID₅₀)

TABLE 2 Half-life estimates for SVA in different feed ingredients incubated at different temperatures

| Treatment | Half-life (days) | 95% confidence interval |
|---------------------------|------------------|-------------------------|
| SBM 4°C | 9.8 | 8.3–11.9 |
| SBM 15°C | 3.0 | 2.4–4.1 |
| SBM 30°C | 1.1 | 0.9–1.5 |
| DDGS 4°C | 25.6 | 20.6–33.7 |
| DDGS 15°C | 8.3 | 5.7–15.5 |
| DDGS 30°C | 0.5 | 0.4–0.7 |
| Lysine 4°C | 1.0 | 0.5 |
| Control 4°C ^a | 1.3 | 1.0–2.0 |
| Control 15°C ^a | 0.5 | 0.4–0.6 |

^aVirus inoculum placed in plastic tubes with no feed matrix.

3.2 | Half-life of SVA in feed ingredients and effect of temperature on virus survival

Half-life estimates were calculated for three feed ingredients and the control at the three treatment temperatures. Due to the rapid virus decay in vitamin D, which resulted in a single time point with measurable viable virus, calculation of half-lives in this ingredient was not performed. Similarly, it was not possible to estimate half-lives of SVA in lysine at 15 and 30°C and in the control samples at 30°C. The virus half-life in SBM and DDGS incubated at 4°C resulted in the longest half-lives of all treatments: 9.8 and 25.6 days, respectively (Table 2). When these two feed ingredients were incubated at 4°C, it also led to the longest estimated time for the virus to decay to 0.01% of the original amount of virus spiked in the ingredients: 126.9 days for SBM and 332.5 days for DDGS. Although SVA was inactivated within 14 days in lysine at 4°C, the half-life in this ingredient was estimated to be 1 day.

3.3 | Detection of nucleic acid

The stability of SVA RNA was also assessed in the feed ingredients following incubation at 4, 15 and 30°C. The viral RNA remained stable in the presence of the feed ingredients throughout the experiment. Interestingly, even in the presence of lysine, where SVA infectivity was

rapidly lost (Figure 2), the virus nucleic acid was steadily detected in all three temperature conditions (Figure 3). Notably, in the absence of the feed matrices (control tube) marked degradation of SVA RNA was observed, with the number of viral genome copies decreasing with the increase in the temperature of incubation (Figure 3).

3.4 | Infection of pigs through contaminated feed

The ability of SVA to infect pigs following ingestion of contaminated feed was evaluated. Animals were monitored and serum samples and rectal swabs were collected to determine the infection status of each animal. Levels of viremia were monitored in serum, whereas virus shedding was monitored in faeces. Additionally, viral load was also assessed in the tonsil collected at necropsy performed at the end of the experiment on day 14.

Viral RNA was detected in faeces and tonsil of all animals in the three experimental groups (10^5 , 10^6 and 10^7) (Figure 4). Viremia was detected in all animals exposed to the higher oral dose 10^7 , whereas two animals in the 10^6 group were viraemic throughout the 14-day experimental period (Figure 4a). In the group exposed to the lower dose (10^5 TCID₅₀), three animals were viraemic in the last day of the experiment. High levels of virus neutralizing antibody titres were detected in all animals from the three treatment groups on day 14 (Figure 4d). These results indicate that consumption of feed contaminated with SVA led to productive virus infection and replication in pigs.

4 | DISCUSSION

The data presented here shows that feed matrices extend the survival and half-life of SVA. Additionally, consumption of feed contaminated with SVA leads to productive infection in pigs. Although previous studies have investigated the stability of SVA in feed matrices, this is the first study assessing its stability under different temperatures for more than 37 days. The prolonged storage time of 91 days at 4°C allowed us to estimate the half-life of SVA in SBM and DDGS. These two feed ingredients protected SVA from degradation, allowing the virus to remain infective for longer periods when compared to vitamin D and lysine, or the control samples in the absence of a feed matrix.

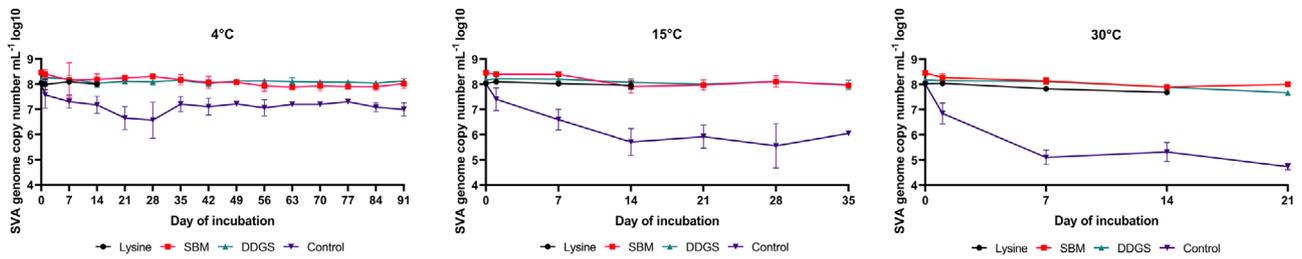


FIGURE 3 SVA genome stability kinetics in animal feed ingredients under different temperatures determined by RT-qPCR. The amount of viral RNA detected in samples was expressed as the log₁₀ (genome copy number)/ml

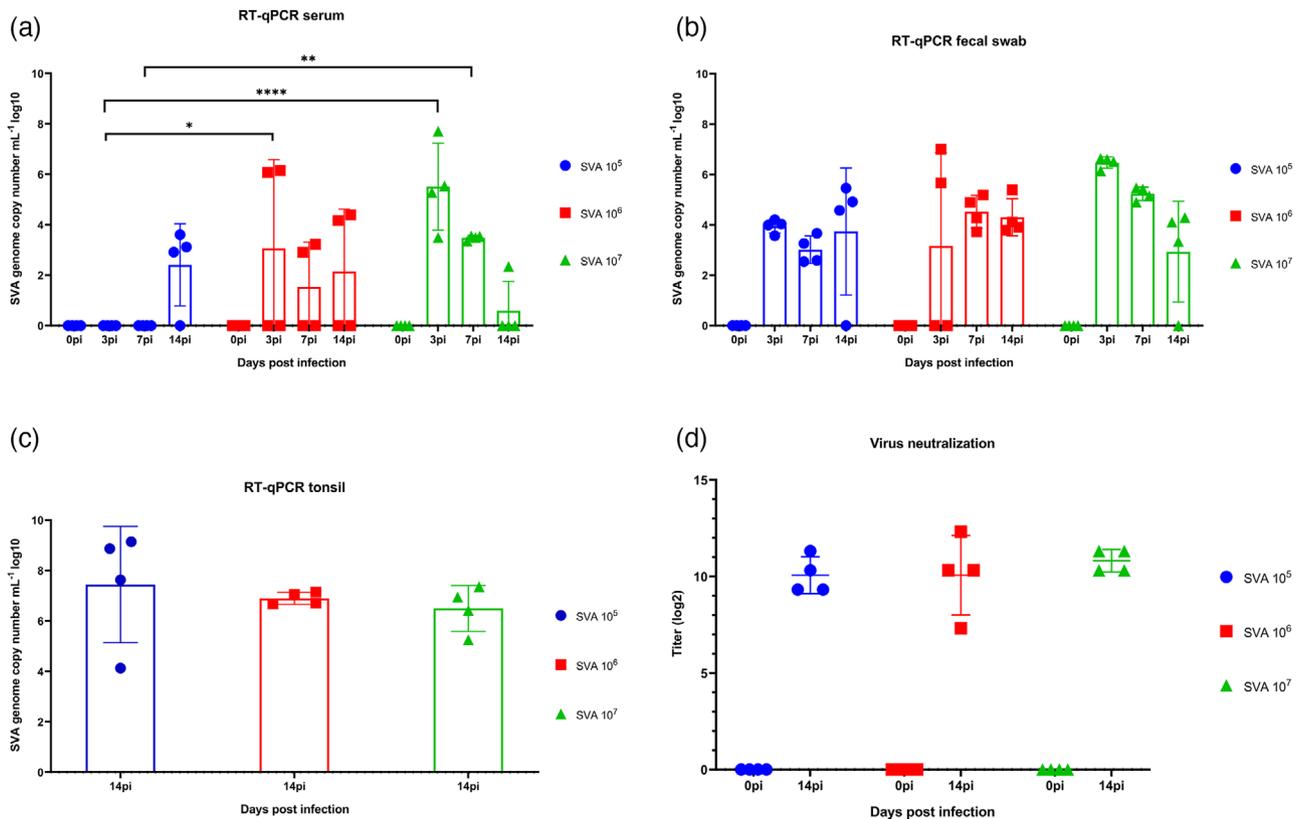


FIGURE 4 Viral load and neutralizing antibodies levels after bioassay with piglets. Viral load in serum (a), faecal swabs (b) and tonsil (c) were determined by RT-qPCR and neutralizing antibodies levels were determined by virus neutralization assay (d) after the consumption of feed contaminated with 10⁵, 10⁶ and 10⁷ TCID₅₀ of SVA per 50 g of feed. Asterisks indicate significant differences: **P* = .0221; ***P* = .0083; *****P* < 0.0001

Both ingredients also favored survival of PRRSV, an enveloped virus, for 37 days under conditions simulating transboundary shipment of feed (Dee et al., 2018). Interestingly, vitamin D did not enable survival of SVA for more than 1 day in the present study, which precluded calculations on the half-life of the virus in this ingredient. The short viability of SVA in vitamin D observed in the present study contrast with our previous findings, in which SVA remained viable until day 37 of incubation in this ingredient (Dee et al., 2018). A possible explanation for these differences could be the composition of carrier compound(s) mixed with vitamin D used in the studies, as the ingredient was sourced from different manufacturers.

Similar to findings of previous reports that assessed the stability of viruses in feed and measured the presence of viral nucleic acid over time (Dee et al., 2018; Stoian et al., 2019, 2020), SVA RNA was unaffected by temperature and remained stable over time in all feed ingredients evaluated. Interestingly, when the virus was exposed to the different range of temperatures in an empty tube 'non-protected' by the feed matrices, marked viral RNA degradation was observed. This was more pronounced when samples were incubated at 15 and 30°C. Although detection of SVA RNA does not reflect the actual infectivity of the sample, the ability to detect viral nucleic acid in feed ingredients for such long periods highlights the fact that testing of feed

ingredients prior to ration formulation or prior to introduction of the feed into farms can potentially be used to prevent the entry of infectious pathogens into swine populations. Several studies have indeed shown that RT-PCR or PCR positive feed samples that are negative on virus isolation can remain infective to pigs, as demonstrated by viral infectivity in swine bioassays (Dee et al., 2016, 2018; Stoian et al., 2020).

When compared to other swine viruses, SVA was shown to be the most stable virus in feed and remained viable for up to 37 days in 10 of 12 feed ingredients tested (Dee et al., 2018). In the present study, the effect of temperature on SVA infectivity was assessed. Notably, although temperature did not significantly affect viral nucleic acid detection by rRT-PCR, virus infectivity was quickly reduced with increased temperatures. These results suggest that perhaps the high protein content of SBM and DDGS, for example, may provide a protective environment that prevents disintegration of the virus capsid (even in temperatures of up to 30°C), and consequent release of RNase-sensitive viral RNA (Howard L. Bachrach, 1964). High temperatures may, however, lead to destabilization of the virus capsid which results in loss of infectivity, without affecting the integrity of the viral RNA. This has indeed been demonstrated for FMDV, a closely related picornavirus, in which heat treatment led to capsid destabilization and loss of infectivity without affecting the integrity of the virus RNA (Bachrach, 1961).

In addition to demonstrating the stability of SVA in feed ingredients, we also performed an *in vivo* experiment to assess SVA infectivity through oral consumption of contaminated feed. The infectivity of a virus through feed depends on its stability, and must consider length and temperature of transportation and storage, and the oral infectious dose of the pathogen. Since our main goal was to determine infectivity of SVA through feed, we spiked feed with three doses of the virus (10^5 , 10^6 and 10^7 TCID₅₀), and stored the feed at 4°C for 2 days prior to feeding it to the animals. The higher dose of 10^7 was selected based on our previous studies, demonstrating successful infection in 100% of inoculated pigs following oronasal inoculation of liquid viral suspensions containing 10^{7-8} TCID₅₀ of SVA (Fernandes et al., 2018; Joshi, et al., 2016; Maggioli et al., 2018). The 10^6 dose was selected as an intermediate dose, whereas the low 10^5 dose was selected based on the dose used to spike feed and feed ingredients here and in most *in vitro* studies demonstrating survival of SVA and other viral pathogens in feed (Dee et al., 2018; Stoian et al., 2020). Results from the studies here demonstrate successful infection with SVA in all groups, with most animals presenting viremia and virus shedding in faeces. Most importantly, all exposed animals presented SVA RNA in the tonsil on day 14 of the experiment and seroconverted to the virus, confirming successful infection through consumption of contaminated feed.

Given that SVA contaminated feed was stored at 4°C prior to feeding and based on our results demonstrating high stability of the virus under this temperature condition (Figure 3), we do not expect that major drops in viral infectivity have occurred during the 48-h incubation period. Therefore, the animals were most likely exposed to infectious viral titres that are very close to the intended target doses.

Unfortunately, back titration of the feed extract was not possible as the relatively large volume of feed precluded us from processing the sample to recover the spiked virus. Nevertheless, results of these studies demonstrate that SVA has a relatively low infectious dose when animals are exposed through oral consumption via contaminated feed, as exposure to 10^5 TCID₅₀ for three consecutive days led to infection of four out of four pigs. Interestingly, a study with African Swine Fever virus (ASFV), another pathogen that is highly stable in feed (Dee et al., 2018), revealed a significant difference in the infectious dose of the virus when given to pigs in a liquid vehicle or in feed (ID₅₀ of 10^1 in liquid vs. $10^{6.8}$ in feed) (Niederwerder et al., 2019). Although we have not assessed the ID₅₀ of SVA in feed in the present study, our results suggest that it is markedly lower than for ASFV. If we take into account the half-life of SVA (Table 2) and the amount of virus recovered from certain ingredients and complete feed (Dee et al., 2018), it is reasonable to expect that animals that would have contact with naturally contaminated feed in the field could potentially be infected and transmit the virus to other animals in the herd. Our results add to the mounting body of evidence that demonstrates that feed matrices increase pathogen stability retaining their infectivity to levels that could lead to productive infection and subsequent transmission in the field.

ACKNOWLEDGEMENTS

We would like to thank the animal care takers at Pipestone Applied Research, for excellent help and care with animal experiments. The work was funded by Swine Health Information Center (SHIC project numbers 18-137 and 18-211).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Scott Dee  <https://orcid.org/0000-0001-8691-3887>

REFERENCES

- Arzt, J., Bertram, M. R., Vu, L. T., Pauszek, S. J., Hartwig, E. J., Smoliga, G. R., Palinski, R., Stenfeldt, C., Fish, I. H., Hoang, B. H., Phuong, N. T., Hung, V. V., Vu, P. P., Dung, N. K., Dong, P. V., Tien, N. N., & Dung, D. H. (2019). First detection and genome sequence of Senecavirus A in Vietnam. *Microbiology Resource Announcements*, 8, e01247-18. <https://doi.org/10.1128/mra.01247-18>
- Bachrach, H. L. (1961). Thermal degradation of foot-and-mouth disease virus into infectious ribonucleic acid. *Proceedings of the Society for Experimental Biology and Medicine*, 107(3), 610-613. <https://doi.org/10.3181/00379727-107-26703>

- Bachrach, H. L. (1964). Foot-and-mouth disease virus: Structure and mechanism of degradation as deduced from absorbance-temperature relationships. *Journal of Molecular Biology*, 8(3), 348–358. [https://doi.org/10.1016/S0022-2836\(64\)80198-4](https://doi.org/10.1016/S0022-2836(64)80198-4)
- Bryan, M., Zimmerman, J. J., & Berry, W. J. (1990). The use of half-lives and associated confidence intervals in biological research. *Veterinary Research Communications*, 14, 235–240. <https://doi.org/10.1007/BF00347743>
- Chen, Z., Yuan, F., Li, Y., Shang, P., Schroeder, R., Lechtenberg, K., Henningson, J., Hause, B., Bai, J., Rowland, R. R. R., Clavijo, A., & Fang, Y. (2016). Construction and characterization of a full-length cDNA infectious clone of emerging porcine Senecavirus A. *Virology*, 497, 111–124. <https://doi.org/10.1016/j.virol.2016.07.003>
- Dee, S., Bauermann, F. V., Niederwerder, M. C., Singrey, A., Clement, T., De Lima, M., Long, C., Patterson, G., Sheahan, M. A., Stoian, A. M. M., Petrovan, V., Jones, C. K., De Jong, J., Ji, J., Spronk, G. D., Minion, L., Christopher-Hennings, J., Zimmerman, J. J., Rowland, R. R. R., Nelson, E., Sundberg, P., & Diel, D. G. (2018). Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PLoS ONE*, 13, e0194509. <https://doi.org/10.1371/journal.pone.0194509>
- Dee, S., Clement, T., Schelkopf, A., Nerem, J., Knudsen, D., Christopher-Hennings, J., & Nelson, E. (2014). An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naive pigs following consumption via natural feeding behavior: Proof of concept. *BMC Veterinary Research*, 10, 176. <https://doi.org/10.1186/s12917-014-0176-9>
- Dee, S., Neill, C., Singrey, A., Clement, T., Cochrane, R., Jones, C., Patterson, G., Spronk, G., Christopher-Hennings, J., & Nelson, E. (2016). Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. *BMC Veterinary Research*, 12, 51. <https://doi.org/10.1186/s12917-016-0674-z>
- Fernandes, M. H. V., Maggioli, M. F., Joshi, L. R., Clement, T., Faccin, T. C., Rauh, R., Bauermann, F. V., & Diel, D. G. (2018). Pathogenicity and cross-reactive immune responses of a historical and a contemporary Senecavirus A strains in pigs. *Virology*, 522, 147–157. <https://doi.org/10.1016/j.virol.2018.06.003>
- Guo, B., Piñeyro, P. E., Rademacher, C. J., Zheng, Y., Li, G., Yuan, J., Hoang, H., Gauger, P. C., Madson, D. M., Schwartz, K. J., Canning, P. E., Arruda, B. L., Cooper, V. L., Baum, D. H., Linhares, D. C., Main, R. G., & Yoon, K. - J. (2016). Novel Senecavirus A in swine with vesicular disease, United States, July 2015. *Emerging Infectious Diseases*, 22, 1325–1327. <https://doi.org/10.3201/eid2207.151758>
- Joshi, L. R., Fernandes, M. H. V., Clement, T., Lawson, S., Pillatzki, A., Resende, T. P., Vannucci, F. A., Kutish, G. F., Nelson, E. A., & Diel, D. G. (2016). Pathogenesis of Senecavirus A infection in finishing pigs. *Journal of General Virology*, 97, 3267–3279. <https://doi.org/10.1099/jgv.0.000631>
- Joshi, L. R., Mohr, K. A., Clement, T., Hain, K. S., Myers, B., Yaros, J., Nelson, E. A., Christopher-Hennings, J., Gava, D., Schaefer, R., Caron, L., Dee, S., & Diel, D. G. (2016). Detection of the emerging picornavirus Senecavirus A in pigs, mice, and houseflies. *Journal of Clinical Microbiology*, 54, 1536–1545. <https://doi.org/10.1128/JCM.03390-15>
- Leme, F. M. M., Dall Agnol, A. M., Alfieri, A. F., & Alfieri, A. A. (2019a). A new wave of Seneca Valley virus outbreaks in Brazil. *Transboundary and Emerging Diseases*, 66, 1101–1104. <https://doi.org/10.1111/tbed.13151>
- Leme, F. M. M., Dall Agnol, A. M., Alfieri, A. F., & Alfieri, A. A. (2019b). Seneca Valley virus RNA detection in pig feed and feed ingredients in Brazil. *Transboundary and Emerging Diseases*, 66, 1449–1453. <https://doi.org/10.1111/tbed.13215>
- Leme, R. A., Alfieri, A. F., & Alfieri, A. A. (2017). Update on senecavirus infection in pigs. *Viruses*, 9, 1–15. <https://doi.org/10.3390/v9070170>
- Maggioli, M. F., Lawson, S., de Lima, M., Joshi, L. R., Bauermann, F. V., & Diel, D. G. (2018). Adaptive immune responses following Senecavirus A infection in pigs. *Journal of Virology*, 92(3), e01717-17. <https://doi.org/10.1128/JVI.01717-17>
- Montiel, N., Buckley, A., Guo, B., Kulshreshtha, V., VanGeelen, A., Hoang, H., Rademacher, C., Yoon, K. J., & Lager, K. (2016). Vesicular disease in 9-week-old pigs experimentally infected with senecavirus A. *Emerging Infectious Diseases*, 22, 1246–1248. <https://doi.org/10.3201/eid2207.151863>
- Niederwerder, M. C., & Rowland, R. R. R. (2017). Is there a risk for introducing porcine reproductive and respiratory syndrome virus (PRRSV) through the legal importation of pork? *Food and Environmental Virology*, 9, 1–13. <https://doi.org/10.1007/s12560-016-9259-z>
- Niederwerder, M. C., Stoian, A. M. M., Rowland, R. R. R., Dritz, S. S., Petrovan, V., Constance, L. A., Gebhardt, J. T., Olcha, M., Jones, C. K., Woodworth, J. C., Fang, Y., Liang, J., & Hefley, T. J. (2019). Infectious dose of African swine fever virus when consumed naturally in liquid or feed. *Emerging Infectious Diseases*, 25(5), 891–897. <https://doi.org/10.3201/eid2505.181495>
- Oļševskis, E., Guberti, V., Seržants, M., Westergaard, J., Gallardo, C., Rodze, I., & Depner, K. (2016). African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Research in Veterinary Science*, 105, 28–30. <https://doi.org/10.1016/j.rvsc.2016.01.006>
- Pasick, J., Berhane, Y., Ojkic, D., Maxie, G., Embury-Hyatt, C., Swekla, K., Handel, K., Fairles, J., & Alexandersen, S. (2014). Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. *Transboundary and Emerging Diseases*, 61(5), 397–410. <https://doi.org/10.1111/tbed.12269>
- Saeng-chuto, K., Rodtian, P., Temeeyasen, G., Wegner, M., & Nilubol, D. (2018). The first detection of Senecavirus A in pigs in Thailand, 2016. *Transboundary and Emerging Diseases*, 65, 285–288. <https://doi.org/10.1111/tbed.12654>
- Sharma, B., Fernandes, M. H. V., de Lima, M., Joshi, L. R., Lawson, S., & Diel, D. G. (2019). A novel live attenuated vaccine candidate protects against heterologous Senecavirus A challenge. *Frontiers in Immunology*, 10, 2660. <https://doi.org/10.3389/fimmu.2019.02660>
- Stoian, A. M. M., Petrovan, V., Constance, L. A., Olcha, M., Dee, S., Diel, D. G., Sheahan, M. A., Rowland, R. R. R., Patterson, G., & Niederwerder, M. C. (2020). Stability of classical swine fever virus and pseudorabies virus in animal feed ingredients exposed to transpacific shipping conditions. *Transboundary and Emerging Diseases*, 67(4), 1623–1632. <https://doi.org/10.1111/tbed.13498>
- Stoian, A. M. M., Zimmerman, J., Ji, J., Hefley, T. J., Dee, S., Diel, D. G., Rowland, R. R. R., & Niederwerder, M. C. (2019). Half-life of African swine fever virus in shipped feed. *Emerging Infectious Diseases*, 25, 2261–2263. <https://doi.org/10.3201/eid2512.191002>
- Sun, D., Vannucci, F., Knutson, T. P., Corzo, C., & Marthaler, D. G. (2017). Emergence and whole-genome sequence of Senecavirus A in Colombia. *Transboundary and Emerging Diseases*, 64, 1346–1349. <https://doi.org/10.1111/tbed.12669>
- Venkataraman, S., Reddy, S. P., Loo, J., Idamakanti, N., Hallenbeck, P. L., & Reddy, V. S. (2008). Structure of Seneca Valley Virus-001: An oncolytic picornavirus representing a new genus. *Structure*, 16, 1555–1561. <https://doi.org/10.1016/j.str.2008.07.013>
- Wen, X., He, X., Zhang, X., Zhang, X., Liu, L., Guan, Y., Zhang, Y., & Bu, Z. (2019). Genome sequences derived from pig and dried blood pig feed samples provide important insights into the transmission of African swine fever virus in China in 2018. *Emerging Microbes & Infections*, 8, 303–306. <https://doi.org/10.1080/22221751.2019.1565915>
- Wu, Q., Zhao, X., Bai, Y., Sun, B., Xie, Q., & Ma, J. (2017). The first identification and complete genome of Senecavirus A affecting pig with idiopathic vesicular disease in China. *Transboundary and Emerging Diseases*, 64, 1633–1640. <https://doi.org/10.1111/tbed.12557>
- Zell, R., Delwart, E., Gorbalenya, A. E., Hovi, T., King, A. M. Q., Knowles, N. J., Lindberg, A. M., Pallansch, M. A., Palmenberg, A. C., Reuter, G., Simmonds, P., Skern, T., Stanway, G., & Yamashita, T. (2017). ICTV virus taxonomy profile: Picornaviridae. *Journal of General Virology*, 98, 2421–2422. <https://doi.org/10.1099/jgv.0.000911>

Zhai, S. L., Wei, W. K., Sun, M. F., Lv, D. H., & Xu, Z. H. (2019). African swine fever spread in China. *Veterinary Record*, 184, 559. <https://doi.org/10.1136/vr.l1954>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: C. Caserta, L., G. Noll, J. C., Singrey, A., Niederwerder, M. C., Dee, S., Nelson, E. A., & Diel, D. G. (2021). Stability of Senecavirus A in animal feed ingredients and infection following consumption of contaminated feed. *Transboundary and Emerging Diseases*, 1-9. <https://doi.org/10.1111/tbed.14310>