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Evaluating the Distribution of Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1 Inoculated Feed After the Use of Physical or Chemical Mitigants to Flush a Feed Manufacturing Facility

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Summary

Contaminated feed is a route of virus transmission between feed mills and swine farms. To reduce the risk of transmission, an understanding of the virus distribution and mitigation strategies are needed. The objective of this study was to evaluate the distribution of porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV), and Seneca Valley virus 1 (SVV1) inoculated feed in the environment and feed of a feed mill before and after the use of chemical mitigants. A 50-lb batch of feed was run through a mixer and bucket elevator followed by a batch inoculated with PEDV, PRRSV, and SVV1. Following the virus-inoculated batch, a flush treatment of either 1) ground corn (GC); 2) GC + 1.5% liquid formaldehyde (LF; SalCURB LF Liquid, Kemin, Des Moines, IA); 3) GC + 1.5% LF + 25% abrasive material (SalCURB; Shell & Bone Builder, Iowa Limestone Company, Urbandale, IA); 4) double flush – GC + 25% abrasive material followed by GC +1.5% LF (Shell & Bone Builder; SalCURB); or 5) dry formaldehyde (SalCURB F2 Dry, Kemin, Des Moines, IA) was utilized, followed by 3 virus-free batches of complete feed. Feed and environmental samples were collected from each piece of equipment following every batch. Dust samples were collected after manufacturing from the inoculated, flush, and final batches from non-feed contact surfaces. Non-feed contact surfaces were considered

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those where dust would accumulate during manufacturing but would not be included in the final diet. The surfaces included the grates of the mixer, the top of the discharge bin following the bucket elevator, and the floor surrounding the same discharge bin. Samples were analyzed via a triplex PCR at the Kansas State University Veterinary Diagnostic Laboratory. A treatment × batch × location interaction was not observed ($P > 0.05$) in feed or the environment for any of the viruses. A flush treatment × batch interaction was observed for SVV1 where greater quantities of viral RNA ($P < 0.05$) were present in the positive batches and the ground corn flush than in those batches which used chemical mitigants or the post-flush batches. A lower quantity of viral RNA ($P < 0.05$) in dust was observed in the last batch of feed compared to the inoculated batch for all viruses; however, SVV1 RNA was still detectable in the dust following the last batch in all treatments. A batch effect ($P < 0.05$) was observed in all sample matrices for PEDV and PRRSV as viral RNA decreased after the implementation of the flush regardless of treatment. The use of chemical mitigants and the implementation of a flush batch reduced the quantity of viral RNA for PEDV, PRRSV, and SVV1. However, viral presence was still observed in feed and the dust on non-feed contact surfaces which could be a source of contamination if re-introduced into finished feed.

Introduction

Complete feed has been discovered as a potential vector for several swine-specific viruses, such as Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV). In an experimental feed mill setting, PEDV RNA was found in the first batch of feed following the initial contamination[5] but remained on surfaces within the feed mill up to the fourth and final batch.[6] Similarly, African swine fever virus (ASFV) DNA was present in all four batches of feed following the initial contamination[7] and was found to remain stable in the environment up to 180 days after contamination.[8]

To reduce the risk of virus transmission in the feed, multiple mitigation strategies have been evaluated. Flushing, or running an ingredient through the feed manufacturing equipment between batches, can help remove residual virus particles from the equipment and dilute remaining virus concentrations in subsequent batches. A rice hull flush, with or without chemical additives, reduced the presence of PEDV in both the

[5] Schumacher, L. L., R. A. Cochrane, A. R. Huss, J. T. Gebhardt, J. C. Woodworth, C. R. Stark, C. K. Jones, J. Bai, R. G. Main, Q. Chen, J. Zhang, P. C. Gauger, J. M. DeRouchey, R. D. Goodband, M. D. Tokach, and S. S. Dritz. 2018. Feed batch sequencing to decrease the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination during feed manufacturing. J Anim Sci 96:4562-4570. doi: 10.1093/jas/sky320

[6] Schumacher, L. L., A. R. Huss, R. A. Cochrane, C. R. Stark, J. C. Woodworth, J. Bai, E. G. Poulsen, Q. Chen, R. G. Main, J. Zhang, P. C. Gauger, A. Ramirez, R. J. Derscheid, D. M. Magstadt, S. S. Dritz, and C. K. Jones. 2017. Characterizing the rapid spread of porcine epidemic diarrhea virus (PEDV) through an animal food manufacturing facility. PLOS ONE 12:e0187309. doi: 10.1371/journal.pone.0187309

[7] Elijah, C. G., J. D. Trujillo, C. K. Jones, N. N. Gaudreault, C. R. Stark, K. R. Cool, C. B. Paulk, T. Kwon, J. C. Woodworth, I. Morozov, C. Gallardo, J. T. Gebhardt, and J. A. Richt. 2021. Evaluating the distribution of African swine fever virus within a feed mill environment following manufacture of inoculated feed. Ibid. 16:e0256138. doi: 10.1371/journal.pone.0256138

[8] Elijah, C. G., J. Trujillo, C. K. Jones, T. Kwon, C. R. Stark, K. Cool, C. B. Paulk, N. Gaudreault, J. C. Woodworth, I. Morozov, C. Gallardo, J. T. Gebhardt, and J. Richt. 2022. Persistence and Distribution of African Swine Fever Virus in Feed and Feed Mill Environment Over Time After Manufacture of Experimentally Inoculated Feed and Subsequent Manufactured Batches of Feed. J Anim Sci 100(Suppl 2):20-21. doi: 10.1093/jas/skac064.034

flush batch and subsequent batches of feed.[9] However, to our knowledge, the efficacy of different flushes with or without chemical mitigants has not been evaluated for viruses currently endemic in the United States. Therefore, the objective of this study was to evaluate different physical and chemical flushes in order to reduce the viral presence and infectivity of SVV1, PEDV, and PRRSV when contaminated feed is introduced into a feed manufacturing facility.

Procedures

Feed inoculation and sample collection was conducted at the Kansas State University Cargill Feed Safety Research Center (FSRC). Biocontainment was entered 10 separate times, representing 10 inoculation cycles needed to complete two replicates of each flush treatment. All protocols were approved by the Kansas State University Institutional Biosafety Committee (IBC-1636).

Inoculum information

Equal volumes of SVV1 (GenBank: KX7780101.1), PEDV CO-isolate (GenBank KF272920), and PRRSV 1-7-4 were used for feed inoculation. The original stock contained 1×10^8 50% tissue culture infectious dose/mL ($TCID_{50}$/mL) SVV1, 1×10^7 $TCID_{50}$/mL PEDV, and 1×10^8 $TCID_{50}$/mL PRRSV. Viruses were individually packaged into 25 mL aliquots, shipped from South Dakota State University to K-State on dry ice, and stored at -112°F until used in the FSRC. One aliquot of each virus was removed from storage on the day of inoculation, transported to the FSRC, and allowed to thaw at room temperature.

Swine diet

A corn-soybean meal gestation diet was manufactured in meal form at Hubbard Feeds (Beloit, KS). Feed samples were collected from multiple bags after feed delivery and submitted for PCR analysis to confirm SVV1, PEDV, and PRRSV negative status prior to entering the FSRC.

Feed inoculation

Virus aliquots were combined (23 mL each) in 613 mL of phosphate buffer solution (PBS) to achieve a one log reduction prior to viral inclusion in feed. Aliquots of each virus prior to dilution and the combined inoculum were retained for analysis. The 682 mL inoculum, with an approximate concentration of 1×10^7 $TCID_{50}$/mL SVV1, 1×10^6 $TCID_{50}$/mL PEDV, and 1×10^7 $TCID_{50}$/mL PRRSV, was added to 4.8 lb of feed in an 11-lb benchtop stainless steel paddle mixer (Cabela's, Inc., Sidney, NE) and mixed for 5 minutes creating 6 lb of feed with an approximate concentration of 1×10^6 $TCID_{50}$/g SVV1, 1×10^5 $TCID_{50}$/g PEDV, and 1×10^6 $TCID_{50}$/g PRRSV. The contents of the benchtop mixer were added to 44 lb of feed and mixed for 5 minutes for a final approximate concentration of 1×10^5 $TCID_{50}$/g SVV1, 1×10^4 $TCID_{50}$/g PEDV, and 1×10^5 $TCID_{50}$/g PRRSV. The inoculated feed was discharged and underwent manufacturing as previously described.

[9] Gebhardt, J. T., R. A. Cochrane, J. C. Woodworth, C. K. Jones, M. C. Niederwerder, M. B. Muckey, C. R. Stark, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. Bai, P. C. Gauger, Q. Chen, J. Zhang, R. G. Main, and S. S. Dritz. 2018. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on porcine epidemic diarrhea virus cross-contamination during feed manufacturing. Journal of Animal Science 96:4149-4158. doi: 10.1093/jas/sky295

Feed manufacturing

Feed manufacturing equipment was primed with a virus-negative batch of feed (primer batch), followed by feed inoculated with equal quantities of SVV1, PEDV, and PRRSV (positive batch), followed by a flush treatment, and three subsequent batches of virus-negative feed (Sequence 1, 2, and 3). During each batch, feed would be mixed for 5 minutes in a 50-lb mixer (Hayes & Stolz Industrial Manufacturing Co. LLC, Burleson, TX) and discharged at a rate of 10 lb/min into a feed bin. Feed would then be poured into the hopper of a bucket elevator (Universal Industries, Cedar Falls, IA) with 74 buckets (each 7 $in.^3$) at an equal discharge rate (10 lb/min) and conveyed through a downspout into a fresh feed bin.

Flush treatments

Five flush treatments were included in the study, with two replicates of each treatment. Flush treatments were manufactured as described in the "Feed manufacturing" section. The application and composition of each flush treatment were as follows:

1. Ground corn: 50 lb of ground corn (500 microns).
2. Sal CURB: A liquid applicator pump and spray nozzle (Lenze, Aerzen, Germany) was connected to the 50-lb mixer. A pre-measured 0.8-lb aliquot of 10% liquid formaldehyde (Sal CURB LF Liquid, Kemin Industries, Inc., Des Moines, IA) was dispensed onto 50 lb of ground corn. Once dispensed, the ground corn + liquid formaldehyde solution was allowed to mix for 5 minutes.
3. Sal CURB + Bone Builder: A 25% abrasive mixture of 12.5 lb of calcium limestone (Shell and Bone Builder, Fehrway Feeds & Livestock Equipment, Winkler, MB, Canada) and 37.5 lb of ground corn was added to the mixer. A pre-measured 0.8-lb aliquot of 10% liquid formaldehyde (Sal CURB LF Liquid, Kemin Industries, Inc., Des Moines, IA) was dispensed onto the abrasive mixture. Once dispensed, the abrasive mixture + liquid formaldehyde solution was allowed to mix for 5 minutes.
4. Bone Builder, then Sal CURB: The first flush, a 25% abrasive mixture of 12.5 lb of calcium limestone (Shell and Bone Builder, Fehrway Feeds & Livestock Equipment, Winkler, MB, Canada) and 37.5 lb of ground corn, was allowed to mix for 5 minutes. Following the physical abrasive flush, a pre-measured 0.8 lb-aliquot of 10% liquid formaldehyde (Sal CURB LF Liquid, Kemin Industries, Inc., Des Moines, IA) was dispensed onto 50 lb of ground corn. Once dispensed, the ground corn + liquid formaldehyde solution was allowed to mix for 5 minutes.
5. Dry formaldehyde: 50 lb of a 4% dry formaldehyde product (Sal CURB F2 Dry, Kemin Industries, Inc., Des Moines, IA) was added to the mixer and allowed to mix for 5 minutes.

Safety precautions

The current approved application of liquid formaldehyde is 6.5 lb/ton or 0.3% inclusion in the diet per manufacturer instructions. The inclusion used in the liquid formaldehyde treatments was 5 times the recommended levels at 32.5 lb/ton or 1.6% inclusion in the diet. For the researchers' safety, full face respirators (6800 Series, 3M, St. Paul, MN) with disposable cartridges (Multi Gas/Vapor Cartridge/Filter 60926, P100, 3M, St. Paul, MN) were used when either liquid or dry formaldehyde products were imple-

mented. Environmental formaldehyde levels were monitored throughout feed manufacturing of subsequent batches with a formaldehyde meter (ENMET, Ann Arbor, MI).

Feed sample collection

Feed samples were collected from the mixer and the bucket elevator during every batch for a total of 12 feed samples per inoculation cycle (14 feed samples for the double flush treatment). Samples from the mixer and bucket elevator were collected using the cut stream method. During discharge, a 2 oz plastic cup (Great Value Cups, 2 oz, Walmart, Bentonville, AR) was filled; this process was repeated twice more every 90 seconds. The three individual samples from each piece of equipment were combined into one composite sample weighing 50 g in a Whirl-pak bag (Nasco, Fort Atkinson, WI).

Environmental sample collection

Environmental samples were collected from the ribbon of the mixer, the boot of the bucket elevator and a bucket of the bucket elevator during every batch for a total of 18 environmental samples per inoculation cycle (21 environmental samples for the double flush treatment). Environmental samples were taken using a 4- × 4-in. cotton surgical gauze pre-moistened with 5 mL of PBS stored in a 50 mL conical tube prior to sampling. The designated area was swabbed, and the gauze was returned to the conical tube.

Dust sample collection

Dust samples were collected from non-feed contact surfaces, such as the top grate of the mixer, the top lip of the discharge bin following the bucket elevator downspout, and the floor surrounding the bucket elevator discharge bin. Samples were collected following the primer batch, the positive batch, the flush batch, and Sequence 3 for a total of 12 dust samples per inoculation cycle (15 dust samples for the double flush treatment). Accumulated dust was collected onto a fresh index card; approximately 2 mL of dust was placed in a 15 mL conical tube for PCR analysis, with remaining dust (between 2–12 mL) retained in a separate conical tube for a bioassay.

Sample processing

All samples were disinfected out of the FSRC, placed on ice, and transported to a biosafety level-2 laboratory in the K-State Veterinary Diagnostic Laboratory for further processing. Feed samples were transferred to 500 mL high density polyurethane bottles with 200 mL of PBS to create a 20% suspension. Similarly, varying quantities of PBS were added to the dust samples directly in the conical tubes to create a 20% suspension. Feed and dust samples were shaken for approximately 10 sec and stored at 39°F overnight. The next day, 25 mL of the supernatant from the feed samples were transferred to fresh 50 mL conical tubes. The supernatant was centrifuged at 4,000 × g for 10 minutes at 46.5°F. Two 300 µL aliquots were retained for PCR analysis and 20 mL was transferred to a fresh 50 mL conical tube for a bioassay. Dust samples were similarly centrifuged with 200–300 µL aliquots retained for PCR; undiluted dust was already retained for the bioassay. For environmental samples, 25 mL of PBS was added and each was tube vortexed for 10 sec. Environmental samples were allowed to incubate at room temperature for one hour. After one hour, a 20 mL aliquot was transferred to a fresh 50 mL conical tube. Centrifugation and sample allocation followed the feed sample procedures.

Quantitative viral analysis

Samples were analyzed for detection of SVV1, PEDV, and PRRSV using quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR) at the Kansas State University Veterinary Diagnostic Laboratory. First, 50 µL of supernatant was placed in a deep well plate and RNA was extracted using a Kingfisher Flex magnetic particle processor (Fisher Scientific, Pittsburgh, PA) and a MagMAX-96 Viral Isolation Kit (Life Technologies, Grand Island, NY). The final elution volume was reduced to 60 µL, and extracted RNA was stored at -112°F until analyzed for SVV1, PEDV, or PRRSV using a qRT-PCR triplex assay with a maximum cycle threshold of 45. Results were reported as the number of samples considered positive and the cycle threshold (Ct) below 45 at which either SVV1, PEDV, or PRRSV RNA was detected.

Bioassay

A bioassay was conducted at Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) utilizing 12 treatment rooms with 3 mixed sex 10-day old piglets in each room. The day 0 inoculation treatments included 1) negative control; 2) pure virus positive control with an equal volume SVV1, PEDV, and PRRSV diluted to the approximate concentration of the positive batch of feed; 3) feed samples from the positive batch; 4) dust samples from the positive batch; 5) feed from the ground corn sequence 1 batch; 6) feed from the ground corn sequence 3 batch; 7) feed from the SalCURB sequence 1 batch; 8) feed from the dry formaldehyde sequence 1 batch; 9) feed from the dry formaldehyde sequence 3 batch; 10) dust from the ground corn sequence 3 batch; 11) dust from the SalCURB sequence 3 batch; and 12) dust from the dry formaldehyde sequence 3 batch. Each pig was inoculated with 2 mL intramuscularly, 2 mL intranasally (1 mL/nostril) and either 10 mL oral gavage (feed samples) or a 10 mL dust slurry fed to the piglet to allow natural swallowing reflexes. Rectal swabs were collected day 1–7 post-inoculation (dpi), blood samples were collected -1, 4, and 7 dpi. Tonsils, lung tissue, jejunal and cecal tissue and cecal contents were collected at necropsy. Day 0 inoculum, rectal (SVV1 and PEDV), and serum (PRRSV) samples were analyzed via PCR at the ISU-VDL.

Statistical analysis

Results were analyzed as a split plot design with the inoculation cycle as the main experimental unit and the sample type (feed, environmental, or dust) from each batch of feed as the sub-plot using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute Inc., Cary, NC). Fixed effects included flush treatment, location, batch, and their associated interactions. Inoculation cycle was included in the model as a random effect. Data were separated and individually analyzed based on the sample type (feed, environmental, or dust) and virus (SVV1, PEDV, and PRRSV). Two response criteria were considered, the number of PCR positive samples and the quantity of detectable viral RNA. Data were analyzed by fitting to a binary distribution, logit link, Laplace approximation, and ridge-stabilized Newton-Raphson algorithm. As a binary distribution model, data were fit by each individual interaction, starting with the treatment × location × batch interaction, and their subsequent main effects. To estimate the quantity of detectable viral RNA, the Ct of each sample was used. If no viral RNA was detected, samples were assigned a value of 45. A Kenward-Roger denominator degree of freedom adjustment was used, as well as a Tukey-Kramer multiple comparison adjustment. Results were considered significant at $P \leq 0.05$.

Results and Discussion

There was no evidence of a treatment × batch × location interaction ($P > 0.05$) for any of the sampling matrices (feed, environmental, or dust) regardless of the virus analyzed. A treatment × batch interaction was observed for SVV1 feed samples (Table 1), where the positive batch had the greatest quantity of viral RNA ($P < 0.05$) and feed samples from the ground corn flush had more detectable SVV1 RNA than either the Sal CURB or dry formaldehyde flush. Similarly, detectable SVV1 RNA was greatest in the positive batch dust samples, with the ground corn flush having the greatest quantity of viral RNA ($P < 0.05$) compared to all other flush treatments (Table 1). There were significantly more ($P < 0.05$) SVV1 positive environmental samples in the two ground corn inoculation cycles (20/36) than in the dry formaldehyde cycles (6/36), with all other flush treatments intermediate. The mixer, a metal surface, had less detectable SVV1 RNA and fewer PCR positive environmental samples ($P < 0.05$) than the plastic bucket within the bucket elevator, with the metal boot of the bucket elevator intermediate. A batch effect was observed for all viruses in each of the sampling matrices (Table 2), except for the PRRSV environmental sample, as no viral RNA was detected in any environmental sample. The positive batch consistently had greater quantities of the respective viral RNA ($P < 0.05$) which decreased with subsequent batches. However, viral RNA was still present in the final batch for SVV1 feed, environmental, and dust samples and PEDV feed samples.

Results of the swine bioassay are found in Table 3. Pigs inoculated with the pure virus positive control showed no signs of shedding PEDV. Pure virus, feed, and dust positive controls were able to cause infection and shedding for both SVV1 and PRRSV. No other treatments displayed signs of SVV1 shedding. PRRSV viremia was noticed in all feed and dust treatment rooms except the dry formaldehyde sequence 3 feed room and ground corn sequence 3 dust room. Interestingly, only two ground corn sequence 3 dust inoculums were PCR positive at either the K-State- or ISU-VDL using PCR, with all other feed or dust treatment inoculums being PCR negative. These results highlight the sensitivity and necessity for swine bioassays to better understand infectivity compared to laboratory assay such as PCR.

Flush batches between the purposefully inoculated batch and the subsequent virus-free batches of feed were able to reduce viral RNA regardless of treatment type. Chemical mitigants reduced viral RNA, specifically SVV1 RNA, more quickly than the non-chemical ground corn treatment; however viral RNA was still present in feed and within the environment after the final batch. The flush treatments were able to reduce SVV1 infectivity of the subsequent feed and dust samples when given to pigs in a bioassay, but non-detectable PRRSV RNA was still infectious when consumed by pigs. Flushing feed manufacturing systems following a pathogen introduction can reduce the initial contamination but did not eliminate the risk of recontamination in both the feed and the environment.

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Table 1. Effect of treatment and batch on the relative quantification of Seneca Valley virus 1 (SVV1) in feed and dust[1]

	Batch					
	Primer	**Positive**	**Flush**	**Sequence 1**	**Sequence 2**	**Sequence 3**
Feed[2]						
Ground corn	45.0	27.8^{c}	34.1^{bc}	38.9^{ab}	45.0^{a}	41.3^{a}
Sal CURB	45.0	27.1^{c}	43.6^{a}	45.0^{a}	45.0^{a}	45.0^{a}
Sal CURB + Bone Builder	45.0	27.7^{c}	40.5^{ab}	43.4^{a}	43.3^{a}	45.0^{a}
Bone Builder, then Sal CURB	45.0	27.3^{c}	39.8^{ab}	45.0^{a}	45.0^{a}	43.3^{a}
Dry formaldehyde	45.0	27.2^{c}	45.0^{a}	45.0^{a}	45.0^{a}	43.5^{a}
Dust[3,4]						
Ground corn	45.0	30.3^{e}	31.9^{de}	—	—	37.6^{bcd}
Sal CURB	45.0	31.6^{de}	42.2^{abc}	—	—	43.5^{ab}
Sal CURB + Bone Builder	45.0	29.8^{e}	38.1^{abcd}	—	—	43.4^{ab}
Bone Builder, then Sal CURB	45.0	28.0^{e}	37.3^{cd}	—	—	43.8^{ab}
Dry formaldehyde	45.0	29.1^{e}	45.0^{a}	—	—	43.8^{ab}

[1] A 50-lb batch of feed was inoculated with SVV1, porcine epidemic diarrhea virus, and porcine reproductive and respiratory syndrome virus, followed by a flush treatment and three virus-negative batches of feed. Feed, environmental, and dust samples were taken following each batch of feed. A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.

[2] Feed Ct value: Treatment × batch, $P = 0.011$, SEM = 1.90

[3] Dust Ct value: Treatment × batch, $P = 0.0004$, SEM = 2.00

[4] Dust samples were not collected during Sequence 1 or 2 feed batches.

^{abcde} means with differing superscripts within matrix differ significantly, $P < 0.05$.

Table 2. Effect of batch on the relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in feed, environmental samples, and dust samples[1]

	Batch							
	Primer	Positive	Flush	Sequence 1	Sequence 2	Sequence 3	SEM	*P* =
SVV1								
Feed samples	45.0	27.5^{c}	40.6^{b}	43.5^{a}	44.7^{a}	43.6^{a}	0.60	< 0.0001
Environmental samples	45.0	33.6^{c}	41.1^{b}	42.6^{ab}	43.4^{a}	44.0^{a}	0.84	< 0.0001
Dust samples[2]	45.0	29.8^{c}	38.7^{b}	—	—	42.4^{a}	0.97	< 0.0001
PEDV								
Feed samples	45.0	36.5^{b}	44.8^{a}	45.0^{a}	45.0^{a}	44.9^{a}	0.47	< 0.001
Environmental samples	45.0	42.8^{b}	44.8^{a}	45.0^{a}	45.0^{a}	45.0^{a}	0.35	< 0.0001
Dust samples[2]	45.0	38.7^{b}	44.7^{a}	—	—	45.0^{a}	0.62	< 0.0001
PRRSV								
Feed samples	45.0	41.3^{b}	44.8^{a}	45.0^{a}	45.0^{a}	45.0^{a}	0.38	< 0.001
Environmental samples[3]	45.0	45.0	45.0	45.0	45.0	45.0	—	—
Dust samples[2]	45.0	43.2^{b}	44.8^{a}	—	—	45.0^{a}	0.36	0.0002

[1] A 50-lb batch of feed was inoculated with SVV1, PEDV, and PRRSV, followed by a flush treatment and three virus-negative batches of feed. Feed, environmental, and dust samples were taken following each batch of feed. A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.

[2] Dust samples were not collected during Sequence 1 or 2 feed batches.

[3] No statistical analysis was conducted for the PRRSV environmental samples since there was no variation between the samples. All Ct values were equal to 45.0 as all samples were negative.

[abc] means with differing superscripts within row differ significantly $P < 0.05$.

Table 3. Effects of physical or chemical flushes as Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) mitigation as evaluated by a swine bioassay[1]

	SVV1		PEDV		PRRSV	
	4 dpi	7 dpi	4 dpi	7 dpi	4 dpi	7 dpi
Control treatments						
Negative	– – –	– – –	– – –	– – –	– – –	– – –
Pure virus	+ + +	+ – –	– – –	– – –	+ + +	+ + +
Feed	– – –	+ + +	– – –	– – –	+ + –	– – –
Dust	+ – –	+ + +	– – –	– – –	+ – –	+ + –
Feed treatments						
Ground corn – Sequence 1	– – –	– – –	– – –	– – –	+ – –	– – –
Ground corn – Sequence 3	– – –	– – –	– – –	– – –	+ + +	+ – –
Sal CURB – Sequence 1	– – –	– – –	– – –	– – –	– – –	+ – –
Dry formaldehyde – Sequence 1	– – –	– – –	– – –	– – –	+ + –	– – –
Dry formaldehyde – Sequence 3	– – –	– – –	– – –	– – –	– – –	– – –
Dust treatments						
Ground corn – Sequence 3	– – –	– – –	– – –	– – –	– – –	– – –
Sal CURB Sequence 3	– – –	– – –	– – –	– – –	+ – –	– – –
Dry formaldehyde – Sequence 3	– – –	– – –	– – –	– – –	+ – –	– – –

[1] Three pigs per each treatment were inoculated on day 0 via intramuscular, intranasal, and either oral gavage (feed samples) or a slurry (dust samples) and evaluated for 7 days. Rectal samples were taken daily to evaluate SVV1 and PEDV presence and blood was collected on 4 and 7 days post-inoculation (dpi) to assess PRRSV presence.
+/– corresponds to the viral status of each pig in the treatment room where + pigs signify viral RNA was present for the respective virus and – pigs had no detectable viral RNA.