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Decontamination of a Feed Manufacturing Environment Following Inoculation with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1

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Summary

Feed mill decontamination is difficult because equipment is not designed to be cleaned with water. Alternate strategies may improve a mill's ability to decontaminate in the event of viral contamination. The objective of this experiment was to evaluate different decontamination strategies within a mill following the inoculation of swine feed with porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV), and Seneca Valley virus 1 (SVV1) run through feed manufacturing equipment consisting of a mixer, bucket elevator, corn cleaner, drag conveyor, and distributor. Afterward, decontamination strategies were implemented with environmental samples collected after each step. Strategy treatments included: 1) complete facility decontamination and heating for 48 hours at 60°C; 2) chlorine dioxide application (ProOxine AH, Bio-Cide International, Inc., Norman, OK); 3) organic matter removal using vacuums (Ridge Tool Company, Elyria, OH) and chlorine dioxide application; 4) heat with portable electric heaters for exactly 48 hours; and 5) organic matter removal and heat with portable heaters for exactly 48 h. A swine bioassay was completed to determine the infectivity of each treatment after decontamination. A treatment × decontamination step × location interaction was observed ($P < 0.05$) for SVV1, where less RNA was detected post-treatment compared to post-inoculation following the complete facility decontamination treatment on surfaces including the mixer, corn cleaner, drag conveyor, and flooring ($P < 0.05$) as compared to all other decontamination treatments. Across all treatments, the act of decontamination reduced detectable PEDV ($P < 0.05$) and PRRSV ($P < 0.05$) RNA when compared to samples

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immediately following inoculation, but complete facility decontamination and heating was the only treatment RNA was non-detectable in all locations. Pigs inoculated with samples collected post-treatment showed no evidence of SVV1 or PEDV infection; PRRSV infection was observed in pigs given the chlorine dioxide with and without organic matter removal treatments and the organic matter removal plus heat treatment. Overall, all treatments reduced detectable RNA for all viruses between the inoculation step and the final decontamination step; however, PRRSV particles remained infectious following decontamination.

Introduction

Feed mill decontamination is a difficult challenge due to the specialized equipment within the mill that is not intended for cleaning, especially with water or liquid disinfectants. Applying water to the equipment increases the risk of rust and mold production, affecting the longevity of the equipment and safety of the manufactured feed. In a previous study, in order to eliminate porcine epidemic diarrhea virus (PEDV) following experimental contamination, the feed mill and equipment had to be power washed, cleaned, disinfected, and held at 60°C for 48 hours.[5] As this method is not practical for commercial mills, alternative methods must be evaluated.

Chlorine dioxide and heat using portable heaters are both methods capable of reaching difficult areas within the feed mill and feed manufacturing equipment. Both methods have decreased viral concentrations given an appropriate contact time.[6,7] Therefore, the objective of this study was to evaluate gaseous ClO_2 or heat exposure as alternate decontamination strategies within a mill following the experimental introduction of PEDV, porcine reproductive and respiratory syndrome virus (PRRSV), and Seneca Valley virus 1 (SVV1) contaminated feed using PCR-based techniques and using a swine bioassay.

Procedures

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

Inoculum information

An equal volume of SVV1 (GenBank: KX7780101.1), PEDV CO-isolate (GenBank KF272920), and PRRSV 1-7-4 (GenBank: PP239061) 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

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[6] Totaro, M., F. Badalucco, A. L. Costa, B. Tuvo, B. Casini, G. Privitera, G. B. Menchini Fabris, and A. Baggiani. 2021. Effectiveness of Disinfection with Chlorine Dioxide on Respiratory Transmitted, Enteric, and Bloodborne Viruses: A Narrative Synthesis. Pathogens 10(8):1017.

[7] Trudeau, M. P., H. Verma, F. Sampedro, P. E. Urriola, G. C. Shurson, J. McKelvey, S. D. Pillai, and S. M. Goyal. 2016. Comparison of Thermal and Non-Thermal Processing of Swine Feed and the Use of Selected Feed Additives on Inactivation of Porcine Epidemic Diarrhea Virus (PEDV). PLoS One 11(6):e0158128. doi: 10.1371/journal.pone.0158128

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 the FSRC, and allowed to thaw at room temperature.

Swine diet

A corn-soybean meal mash gestation diet was manufactured 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

Viruses were combined (23 mL each) in 613 mL of phosphate buffer solution (PBS) to create an adequate volume for mixing in 22.7 kg of feed. Aliquots of each virus prior to dilution and the combined viruses with PBS inoculum were retained for analysis. The 682 mL total inoculum was first added to 4.8 lb of feed and mixed by hand for 5 minutes in a 11 lb benchtop stainless steel paddle mixer (Cabela's Inc., Sidney, NE) creating 6 lb of feed. The inoculated feed was added to an additional 44 lb of feed and mixed for another 5 minutes. In total, 23 mL of each virus with an initial concentration of 1×10^8 $TCID_{50}$/mL (PEDV at 1×10^7 $TCID_{50}$/mL) was evenly distrusted in 50 lb of feed which provides an approximate concentration of 1×10^5 $TCID_{50}$/g feed for SVV1 and PRRSV and 1×10^4 $TCID_{50}$/g feed for PEDV.

Feed manufacturing

The feed manufacturing equipment was primed with 100 lb of virus-free feed, utilizing the full capacity of the mixer (model #SS-LI, H.C. Davis Sons Manufacturing Co., Bonner Springs, KS). Feed was mixed for 5 minutes before discharging at a rate of 10 lb/min into a feed bin. Feed was then poured into the hopper of a double-shaft bucket elevator (model B3, Universal Industries, Inc., Cedar Falls, IA) with 3 in × 3.75 in-sized buckets at an equal discharge rate (10 lb/min), which dispensed feed into a corn cleaner (Standard Gentle Roll single drum, EBM Manufacturing, Norfolk, NE) before being deposited into a feed bin. Large particles separated in the corn cleaner were discarded. Feed was transported to the drag conveyor ("RB" Round-Bottom Drag-Flite Standard Direct Inlet, Essmueller Co., St. Louis, MO) and discharged at the same rate of 10 lb/min before being carried up two flights of stairs and poured into the distributor (10 outlets, Model E, Hayes & Stolz Industrial Manufacturing Co., Burleson, TX) and subsequent down spout, which discharged feed into a bin one floor below. The previously described inoculated batch of feed was manufactured in the same manner, but due to the limited quantity of virus stock only 48.7 lb of feed was used for the inoculated batch of feed. Due to the set-up of the FSRC the mixer, bucket elevator, corn cleaner, and distributor were segregated from the rest of the room by a cloth tarp creating their own micro-environment. The distributor was located on the third floor with the connected down spout discharging onto the second floor.

Decontamination treatments

Five decontamination treatments were included in the study, with two replicates of each treatment. Decontamination treatments followed the inoculation batch and were as follows:

1. Complete FSRC decontamination – removal of organic matter with heated pressure washing, disinfection with 1% peroxygen (Virkon S, Lanxess, Cologne Germany), disinfection with 5% bleach solution (7.5% sodium hypochlorite; Clorox, Oakland, CA), environmental heat held at 140°F for 48 hours. The 48-hour heat-up period started once the temperature on all floors reached and maintained the 140°F minimum threshold.
2. ClO_2 – application of a commercial chlorine dioxide product (approximate concentration = 500 ppm; ProOxine AH, Bio-Cide International, Inc., Norman, OK) via a stationary fogger (Automated Activation Non-Electric (AANE) modified for hand mixing product, Bio-Cide International Inc. Norman, OK) and a portable fogger (BCI Atomizer, Bio-Cide International, Inc., Norman, OK). Both foggers operated until empty (approximately 15-20 minutes). Samples were collected one hour after fogger shut-off to allow time for product contact time.
3. Organic matter removal + ClO2 – organic matter was removed with portable vacuums (4-gallon Ridgid portable wet/dry vacuum, Ridge Tool Company, Elyria, OH) from surfaces where feed build-up occurred (bottom of bucket elevator, drag conveyor, mixer, and corn cleaner, flooring, etc.). Commercial chlorine dioxide was applied via a stationary fogger and a portable fogger. Both foggers operated until empty (approximately 15-20 minutes). Samples were collected one hour after fogger shut-off to allow time for product contact time.
4. Heat – two portable electric heaters operated at the highest temperature possible for 48 hours following the inoculation batch of feed. The 48-hour heat period began immediately once the heaters were turned on. The first portable heater (SDRA Series, Chromalox, Inc., Pittsburgh, PA) was in the micro-environment on the first floor. To trap as much heat within the micro-environment, rubber mats were laid on the slated flooring on the second floor to create a ceiling-like structure. The second heater (FES Series, Fostoria Industries, Gray, TN) was located on the second floor and aimed up the downspout into the distributor. Data loggers (HOBO MX temp/RH logger, Onset, Bourne, MA) were placed on all pieces of equipment during heat up near the sampling area.
5. Organic matter removal + heat – organic matter was removed with portable vacuums from surfaces where feed build-up occurred (bottom of bucket elevator, drag conveyor, mixer, and corn cleaner, flooring, etc.). Two portable electric heaters operated at the highest temperature possible for 48 hours following the inoculation batch of feed. The 48-hour heat period began immediately once the heaters were turned on. The first portable heater was in the micro-environment on the first floor. To trap as much heat within the micro-environment, rubber mats were on the slated flooring on the second floor to create a ceiling-like structure. The second heater was located on the second floor and aimed up the downspout into the distributor. Data loggers were placed on all pieces of equipment during heat up near the sampling area.

Safety precautions

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 once chlorine dioxide decontamination was implemented.

Environmental sample collection

Environmental samples were collected from the ribbon of the mixer, the boot and bucket of the bucket elevator, the wall near the bucket elevator (< 3.3 ft from the hopper), the internal side of the corn cleaner, bottom of the drag conveyor, inside of the distributor, bottom of the downspout, the flooring around the downspout where feed was discharged, and the worker's boot. Samples were taken following each feed manufacturing batch (primer and inoculation) and after each decontamination step. Briefly, a 4 in × 4 in cotton surgical gauze pre-moistened with 5 mL of PBS and stored in a 50 mL conical tube prior to sampling was used to swab a designated area, and the gauze was returned to the conical tube.

Sample processing

Upon leaving the FSRC, samples underwent disinfection protocols before being placed on ice and transported to a biosafety level-2 laboratory in the K-State Veterinary Diagnostic Laboratory for further processing. For environmental samples, 25 mL of PBS was added and each tube was vortexed for 10 sec. 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. 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.

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

The experimental bioassay included nine treatment rooms with three 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 inoculation batch of feed, 3) environmental samples from the inoculation batch, 4) environmental samples after organic matter removal with the vacuums, 5) environmental samples following the 48-hour heat up at 60°C from the complete FSRC decontamination, 6) environmental samples taken after ClO_2 application, 7) environmental samples taken after ClO_2 application in the organic matter removal treatment, 8) samples taken after using portable heaters for 48 hours, and 9) samples taken after using portable heaters for 48 hours in the organic matter removal treatment. Each pig was inoculated with 2 mL intramuscularly, 2 mL intranasally (1 mL/nostril) and a 10 mL oral gavage. Prior to piglets arriving at ISU-VDL, sow serum was tested for PRRSV with an enzyme-linked immunosorbent assay and SVA and PEDV with an indirect fluorescent antibody assay. Additionally, sow serum and piglet rectal swabs were tested for PRRSV, SVV1, and enteric coronaviruses via PCR.

Prior to inoculation, individual piglet serum and feces were also tested for PRRSV, SVV1, and enteric coronaviruses via PCR. All sows and piglets tested negative for SVV1, PEDV, and PRRSV prior to inoculation. Rectal swabs were collected day 1-7 post-inoculation, blood samples were collected -1, 4, and 7 dpi. Tonsils, lung tissue, jejunal and cecal tissue and cecal contents were collected at necropsy on d 7 dpi. 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 experimental unit for decontamination treatment, and the environmental samples following the inoculated batch of feed and the final decontamination step as the experimental unit for location and decontamination step using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Fixed effects included decontamination treatment, location, decontamination step (either inoculated batch of feed or final decontamination step), and their associated interactions. Inoculation cycle was included in the model as a random effect. Data were separated and individually analyzed based on virus (SVV1, PEDV, and PRRSV). Contrast statements were included to compare the final decontamination step of the complete FSRC plus heat decontamination to the final decontamination step of each individual treatment (ClO_2, organic matter removal + ClO_2, heat, or organic matter removal + heat). Contrasts statements were also used to compare ClO_2 to the organic matter removal + ClO_2 and heat to organic matter removal + heat. Additionally, the four inoculation cycles, which included organic matter removal via vacuums, were analyzed individually to compare the inoculation batch of feed to the removal of organic matter creating a decontamination step × location interaction and their associated main effects. For all analysis, 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 decontamination treatment × decontamination step × location 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 Ct 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

As expected, viral RNA was not detected on environmental surfaces following the primer batch of feed for the ten inoculation cycles. The average temperature and relative humidity from the environment around each piece of equipment between the four inoculations cycles using portable heaters ranged between 110-125°F and relative humidity between 13.9-20.9%. The average temperature was reached approximately 8-9 hours after the heaters were turned on for the 48-hour period. A decontamination treatment × decontamination step × location interaction was observed for SVV1 ($P < 0.05$) where less RNA was on environmental surfaces post-treatment compared to post-inoculation following complete facility decontamination on surfaces including the mixer, corn cleaner, drag conveyor, and floor around the feed discharge (Table 1). No differences were observed ($P > 0.05$) for the bucket elevator, wall, distributor, down-

spout, or boot between the inoculation step and the final complete facility decontamination step. Interactions were not noted for either PEDV or PRRSV ($P > 0.05$), but greater quantities of RNA ($P < 0.05$) were detected on surfaces following inoculation than after completion of the decontamination treatment for all viruses. Subsequently, fewer PCR positive samples ($P < 0.05$) were detected after the decontamination treatments than after inoculation for SVV1, PEDV, and PRRSV.

Location also affected the quantity of RNA detected, where the wall near the bucket elevator, downspout, and the worker boot had less detectable SVV1 RNA ($P < 0.05$) than the mixer, bucket of the bucket elevator, corn cleaner, drag conveyor, and the flooring around the discharge. Fewer PCR-positive samples were detected ($P < 0.05$) from the locations with the least detectable SVV1 RNA. Greater quantities of PRRSV RNA ($P < 0.05$) were detected from the corn cleaner, drag conveyor, and the floor around the discharge than the wall near the bucket elevator and the worker boot. Similarly, the wall had less detectable PEDV RNA ($P < 0.05$) than the floor around the discharge; however, no differences were observed ($P > 0.05$) for the proportion of PCR positive PRRSV or PEDV samples.

Following the removal of organic matter using vacuums, a decontamination step × location effect was not observed ($P > 0.05$) for any virus. Removing organic matter decreased the quantity of detectable SVV1 and PEDV RNA ($P < 0.05$), decreased the number of PCR-positive PEDV samples ($P < 0.05$), and tended to decrease the quantity of PRRSV RNA ($P < 0.10$). The summary of contrasts statements is shown in table 2. Complete FSRC decontamination plus heat had less detectable SVV1 RNA ($P < 0.05$) than any other treatments, which was similar for PEDV, with the exception of organic matter removal + ClO_2, which did not differ from complete FSRC decontamination plus heat treatment ($P > 0.05$). The heat treatment for PRRSV was the only decontamination strategy that significantly differed ($P < 0.05$) from the complete FSRC decontamination treatment with greater quantities of PRRSV RNA found after using portable heaters for 48 hours. Removing organic matter decreased detectable SVV1 and PEDV RNA ($P < 0.05$) when comparing ClO_2 to its respective organic matter removal counterparts and SVV1 and PRRSV RNA ($P < 0.05$) when comparing the two heat treatments.

The results of the swine bioassay are shown in table 3. As expected, pigs inoculated with samples from the primer batch of feed (virus negative) showed no signs of viral infection. Signs of SVV1 and PRRSV infection were noticed from pigs inoculated with the pure virus, but interestingly, the pure virus failed to cause PEDV infection in pigs. Only SVV1 infection and replication was observed when pigs were inoculated with samples following the inoculated batch of feed. Samples from the decontamination treatments and the removal of organic matter failed to cause SVV1 and PEDV infection in any pigs. Although the inoculums were not consistently PCR positive for PRRSV, infection was observed in every decontamination treatment room except the complete FSRC decontamination and heat-alone treatment. It is unknown why PRRSV RNA was not detected via PCR but was present in great enough quantities to cause infection, but subsequent analysis showed cross-contamination between rooms or infection from a different PRRSV strain to be unlikely.

Complete feed mill decontamination using power washing, disinfectants, and extended heat periods is an effective, but impractical decontamination strategy. The use of chlorine dioxide and portable heaters had limited effect on the quantity of detectable viral RNA but did decrease the risk of infectivity for SVV1 and PEDV; however, PRRSV infection was still observed following these more practical strategies. Overall, chlorine dioxide and portable heaters show promise at reducing the risk of infection when utilized in feed manufacturing facilities, but viral RNA may still be detectable regardless of the decontamination strategy utilized.

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Table 1. Effect of decontamination treatment, decontamination step (inoculation vs post-treatment), and location on the relative quantification of Seneca Valley virus 1 (SVV1) on environmental surfaces following inoculation and the final step of the decontamination protocol.[1]

	Location									
	Mixer	Bucket elevator – boot	Bucket elevator – bucket	Wall near bucket elevator	Corn cleaner	Drag conveyor	Distrib-utor	Down spout	Floor around discharge	Boot
ClO_2[2]										
Inoculation	31.4^{bc}	33.7^{abc}	34.8^{abc}	41.6^{abc}	30.0^{bc}	30.2^{bc}	32.2^{abc}	36.6^{abc}	29.4^{bc}	37.9^{abc}
Post-treatment	31.0^{bc}	36.1^{abc}	35.1^{abc}	45.0^{a}	30.4^{bc}	31.0^{bc}	36.3^{abc}	37.4^{abc}	31.9^{bc}	36.3^{abc}
Organic matter removal + ClO_2[3]										
Inoculation	31.4^{bc}	40.1^{abc}	34.6^{abc}	45.0^{a}	29.9^{bc}	29.9^{bc}	35.3^{abc}	41.0^{abc}	29.6^{bc}	41.1^{abc}
Post-treatment	36.2^{abc}	37.7^{abc}	35.4^{abc}	45.0^{a}	36.1^{abc}	36.2^{abc}	40.6^{abc}	41.8^{abc}	41.2^{abc}	45.0^{a}
Heat[4]										
Inoculation	30.7^{bc}	35.8^{abc}	34.4^{abc}	45.0^{a}	30.0^{bc}	29.7^{bc}	34.2^{abc}	40.2^{abc}	30.2^{bc}	37.8^{abc}
Post-treatment	36.2^{abc}	41.1^{abc}	35.7^{abc}	42.1^{abc}	33.3^{abc}	33.3^{abc}	37.2^{abc}	41.6^{abc}	33.5^{abc}	41.6^{abc}
Organic matter removal + heat[5]										
Inoculation	29.8^{bc}	37.0^{abc}	39.1^{abc}	45.0^{a}	29.8^{bc}	30.3^{bc}	36.4^{abc}	41.0^{abc}	30.2^{bc}	41.2^{abc}
Post-treatment	41.7^{abc}	41.3^{abc}	36.7^{abc}	45.0^{a}	42.1^{abc}	41.7^{abc}	41.7^{abc}	45.0^{a}	38.1^{abc}	45.0^{a}
Complete FSRC decontamination[6]										
Inoculation	29.8^{bc}	36.7^{abc}	36.1^{abc}	45.0^{a}	30.4^{bc}	31.0^{bc}	33.2^{abc}	40.8^{abc}	30.6^{bc}	42.5^{ab}
Post-treatment	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}	45.0^{a}

[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 decontamination protocol. Environmental samples were taken following each decontamination step, but only the samples following inoculation and the final stage in each decontamination protocol are displayed. Values reported are cycle threshold (Ct) and a Ct value of 45.0 is considered negative with no detectable viral RNA. Treatment × decontamination step × location, $P = 0.04$, SEM = 2.92

[2] ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[3] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[4] Portable heaters ran for exactly 48 hours.

[5] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); portable heaters ran for exactly 48 hours.

[6] Removal of organic matter with heated pressure washing, disinfection with 1% peroxygen (Virkon S, Lanxess, Cologne, Germany), disinfection with 5% bleach solution (7.5% sodium hypochlorite; Clorox, Oakland, CA), environmental heat held at 140°F for 48 hours. The 48 hour heat up period was started once temperature on all floors reached and maintained the 60°C minimum threshold.

[abc] means with differing superscripts within matrix differ significantly, $P < 0.05$

Table 2. Summary of treatment contrasts statements on the relative quantification of Seneca valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) and proportion of PCR positive on environmental surfaces following the final step of the decontamination protocol.[1,2]

	Treatment					*P* =	
	ClO_2[3]	Organic matter removal + ClO_2[4]	Heat[5]	Organic matter removal + heat[6]	Complete FSRC decontamination[7]	Ct	Proportion PCR positive
SVV1							
Complete vs ClO_2	35.0 (18/20)	–	–	–	45.0 (0/20)	< 0.0001	0.959
Complete vs Organic removal + ClO_2	–	39.5 (13/20)	–	–	45.0 (0/20)	< 0.0001	0.963
Complete vs Heat	–	–	37.6 (16/20)	–	45.0 (0/20)	< 0.0001	0.961
Complete vs organic removal + heat	–	–	–	41.8 (9/20)	45.0 (0/20)	0.009	0.965
ClO_2 vs Organic removal + ClO_2	35.0 (18/20)	39.5 (13/20)	–	–	–	0.0003	0.076
Heat vs Organic removal + heat	–	–	37.6 (16/20)	41.8 (9/20)	–	0.0005	0.029
PEDV							
Complete vs ClO_2	41.3 (9/20)	–	–	–	45.0 (0/20)	0.0004	0.965
Complete vs Organic removal + ClO_2	–	44.8 (1/20)	–	–	45.0 (0/20)	0.809	0.972
Complete vs Heat	–	–	42.8 (6/20)	–	45.0 (0/20)	0.031	0.967
Complete vs organic removal + heat	–	–	–	42.2 (7/20)	45.0 (0/20)	0.006	0.966
ClO_2 vs Organic removal + ClO_2	41.3 (9/20)	44.8 (1/20)	–	–	–	0.0009	0.016
Heat vs Organic removal + heat	–	–	42.8 (6/20)	42.2 (7/20)	–	0.538	0.737
PRRSV							
Complete vs ClO_2	44.7 (1/20)	–	–	–	45.0 (0/20)	0.404	0.988
Complete vs Organic removal + ClO_2	–	45.0 (0/20)	–	–	45.0 (0/20)	1.00	1.00
Complete vs Heat	–	–	44.2 (3/20)	–	45.0 (0/20)	0.021	0.987
Complete vs organic removal + heat	–	–	–	45.0 (0/20)	45.0 (0/20)	1.000	1.00
ClO_2 vs Organic removal + ClO_2	44.7 (1/20)	45.0 (0/20)	–	–	–	0.404	0.988
Heat vs Organic removal + heat	–	–	44.2 (3/20)	45.0 (0/20)	–	0.021	0.987

[1] A 50 lb batch of feed was inoculated with SVV1, PEDV, and PRRSV, followed by a decontamination protocol. Environmental samples were taken following each decontamination step, but only the samples following the final stage in each decontamination protocol are displayed. A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.

[2] The Ct represents the value averaged across all ten surfaces (mixer, boot of bucket elevator, bucket of bucket elevator, corn cleaner, drag conveyor, distributor, downspout, wall, flooring, and boots) for each decontamination step.

[3] ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[4] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[5] Portable heaters ran for exactly 48 hours.

[6] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); portable heaters ran for exactly 48 hours.

[7] Removal of organic matter with heated pressure washing, disinfection with 1% peroxygen (Virkon S, Lanxess, Cologne Germany), disinfection with 5% bleach solution (7.5% sodium hypochlorite; Clorox, Oakland, CA), environmental heat held at 60°C for 48 hours. The 48-hour heat-up period started once the temperature on all floors reached and maintained the 140°F minimum threshold.

Table 3. Effects of treatment on Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) decontamination as evaluated by a swine bioassay.[1]

	SVV1			PEDV			PRRSV		
	Inoculum Ct (PCR positive inoculum)[2]	4 dpi	7 dpi	Inoculum Ct (PCR positive inoculum)[2]	4 dpi	7 dpi	Inoculum Ct (PCR positive inoculum)[2]	4 dpi	7 dpi
Control treatments									
Negative	(0/3)	– – –	– – –	(0/3)	– – –	– – –	(0/3)	– – –	– – –
Pure virus	23.5 (3/3)	+ + +	+ – –	25.9 (3/3)	– – –	– – –	28.4 (3/3)	+ + +	+ + +
Environmental	26.7 (3/3)	– – –	+ – –	36.8 (3/3)	– – –	– – –	37.9 (3/3)	– – –	– – –
Decontamination treatments[3]									
ClO_2[4]	28.5 (3/3)	– – –	– – –	38.6 (2/3)	– – –	– – –	37.1 (1/3)	+ – –	+ – –
Organic matter removal + ClO_2[5]	37.1 (3/3)	– – –	– – –	(0/3)	– – –	– – –	(0/3)	+ – –	+ – –
Heat[6]	33.2 (3/3)	– – –	– – –	36.2 (2/3)	– – –	– – –	38.3 (2/3)	– – –	– – –
Organic matter removal + heat[7]	38.0 (3/3)	– – –	– – –	39.5 (2/3)	– – –	– – –	(0/3)	– – –	+ – –
Complete FSRC decontamination[8]	(0/3)	– – –	– – –	(0/3)	– – –	– – –	(0/3)	– – –	– – –
Organic matter removal alone[9]	34.9 (3/3)	– – –	– – –	39.2 (1/3)	– – –	– – –	(0/3)	+ + –	+ – –

[1] Three pigs per each treatment were inoculated on day 0 via intramuscular, intranasal, and oral gavage and evaluated for seven days. Rectal samples were taken daily to evaluate SVV1 and PEDV presence and blood was collected on 4 and 7 dpi to assess PRRSV presence.

[2] The cycle threshold (Ct) value is the average Ct of all the PCR positive inoculums within each room as analyzed at Iowa State University Veterinary Diagnostic Laboratory following the d 0 inoculation. If no detectable RNA was present in the inoculum, then it was not included in the average Ct. The numerator represents the number of PCR positive inoculums with the denominator representing the total number of inoculums.

[3] Pigs were inoculated with environmental samples only from the final step of decontamination; no intermediary steps were utilized.

[4] ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[5] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); ProOxine AH (Bio-Cide International, Inc., Norman, OK)

[6] Portable heaters ran for exactly 48 hours.

[7] Organic matter removal with portable vacuums (Ridge Tool Company, Elyria, OH); portable heaters ran for exactly 48 hours.

[8] Removal of organic matter with heated pressure washing, disinfection with 1% peroxygen (Virkon S, Lanxess, Germany), disinfection with with 5% bleach solution (7.5% sodium hypochlorite; Clorox, Oakland, CA), environmental heat held at 60°C for 48 hours. The 48-hour heat-up period started once the temperature on all floors reached and maintained the 60°C minimum threshold.

[9] Pigs were inoculated with the environmental samples following organic matter removal using vacuums decontamination step regardless of the treatment of origin (organic matter removal + ClO_2 or heat), as no treatment was applied when organic matter was removed.

+/– 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.