RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of an accelerated hydrogen peroxide disinfectant and a chlorine dioxide disinfectant to inactivate porcine epidemic diarrhea virus in swine packing plant dock and unloading areas – **NPB - #15-121**

Investigator: Derald Holtkamp[1]

Co-investigators: Kimberlee L. Gerardy[1], Christine Mowrer[1], Locke Karriker[1, 3], Alejandro Ramirez[1, 3], Jianqiang Zhang[1]

Institution: Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University College of Veterinary Medicine, Ames, IA[1]
AMVC Management Services, Audubon, IA[2]
Swine Medicine Education Center, Iowa State University College of Veterinary Medicine, Ames, IA[3]

Date Submitted: March 11, 2016

INDUSTRY SUMMARY

Contaminated swine transport vehicles played a role in the rapid spread of porcine epidemic diarrhea virus (PEDV) across the United States in 2013. One potential source of contamination for livestock trailers is the unloading dock at swine packing plants or harvest facilities. Therefore, quick, practical, low cost methods of swine packing plant unloading dock sanitation need to be developed to prevent possible trailer contamination in the future. The objective of this study was to test the efficacy of an accelerated hydrogen peroxide (AHP) disinfectant and a Chlorine Dioxide (CD) disinfectant at inactivating PEDV in swine feces on concrete surfaces with a very short contact time under both warm (20ºC) and cold (-10ºC) conditions.

PEDV positive feces (PEDV negative feces for the Negative Control group) were spread evenly on a concrete coupon modeled after the non-slip "waffle" flooring commonly found in swine packing plant unloading docks. Eight treatment groups evaluating two concentrations of AHP disinfectant (1:32 and 1:64), two concentrations of CD disinfectant (100 ppm and 50 ppm), and two temperatures (20ºC and -10ºC) were evaluated using a fixed level of fecal contamination (10 ml) and contact time (5 minutes). For the treatment groups evaluated at -10ºC, the AHP and CD disinfectants were mixed into a solution that was 10% propylene glycol (PG), to prevent freezing. A negative control and positive control group were also evaluated. The positive control group was sham disinfected with a sterile water / PG solution. Each treatment group consisted of four replicates (4 concrete coupons and 4 pigs per treatment). After disinfectant application, the contents of the concrete coupons were re-collected and administered to 3-week old pigs through a gastric tube. Each coupon was matched to an

individual pig that served as a swine bioassay to determine if the PEDV was still infectious after disinfectant treatment. Infectivity was determined by detection of PEDV with reverse transcriptase polymerase chain reaction (RT-PCR) quantitation on rectal swabs collected from pigs 3 and 7 days after inoculation. Pigs in each treatment group were housed individually in raised tubs.
The positive control pigs failed to become positive through swine bioassay; therefore, the results from this study are inconclusive and cannot be used to determine the effectiveness of either the AHP or CD disinfectants at inactivating PEDV in swine feces on concrete surfaces.

Contact information for correspondence:
Dr. Derald Holtkamp DVM, MS
Associate Professor, Iowa State University College of Veterinary Medicine
Business: (515) 294-9611
Mobile: (515) 520-1040
2233 Lloyd Veterinary Medical Center
1809 S Riverside Dr.
Ames, IA 50011-1250

KEYWORDS
Swine, PEDV, disinfection, temperature, accelerated hydrogen peroxide

SCIENTIFIC ABSTRACT

Contaminated swine transport vehicles played a role in the rapid spread of porcine epidemic diarrhea virus (PEDV) across the United States in 2013. One potential source of contamination for livestock trailers is the unloading dock at swine packing plants or harvest facilities. These facilities typically do not require employees to perform sanitation and decontamination procedures on the unloading area between livestock trailers. The objective of this study was to test the efficacy of an accelerated hydrogen peroxide (AHP) disinfectant and a Chlorine Dioxide (CD) disinfectant at inactivating PEDV in swine feces on concrete surfaces, representative of those found in swine packing plants, with a very short contact time under both warm (20ºC) and cold (-10ºC) conditions. Ten treatment groups, including a positive control and a negative control group were evaluated. Two concentrations of AHP disinfectant (1:32 and 1:64), two concentrations of CD disinfectant (100 ppm and 50 ppm), and two temperatures (20ºC and -10ºC) were evaluated using a fixed level of fecal contamination (10ml) and contact time (5 minutes). For the treatment groups evaluated at -10ºC, the AHP and CD disinfectants were mixed into a solution that was 10% propylene glycol (PG) by volume, to prevent freezing. The positive control group was conducted at -10ºC and sham disinfected with a sterile water / PG solution. Forty concrete coupons, designed to mimic the non-slip "waffle" flooring present in swine packing plant unloading docks were matched with 40 clinically healthy 3-week old barrows. Each treatment group was conducted in 4 replicates (4 concrete coupons and 4 barrows per treatment). Ten (10) ml of PEDV positive feces (PEDV negative feces for the Negative Control group) were applied evenly to the concrete coupon. Feces were allotted a 10 minute rest period after application and then the subjected to one of the ten treatment groups described above. Following treatment, the contents from the coupons were suspended in sterile saline, collected, and administered to pigs via oral gavage. These pigs served as a bioassay to determine the infectivity of PEDV after treatment with either the AHP or CD disinfectant. Pigs were housed individually in raised tubs and observed for 7 days post

inoculation. PEDV infectivity was determined by the detection of PEDV ribonucleic acid (RNA) by reverse transcriptase polymerase chain reaction (RT-PCR) quantitation on rectal swabs collected from pigs 3 and 7 days after inoculation. The positive control pigs failed to become positive through swine bioassay; therefore, the results from this study are inconclusive and cannot be used to determine the effectiveness of either the AHP or CD disinfectants on inactivating PEDV in swine feces on concrete surfaces.

INTRODUCTION

In May of 2013, porcine epidemic diarrhea virus (PEDV) was identified in swine in the United States. The virus caused severe diarrhea in sows and piglets, with near 100% mortality in piglets across a wide geographical area of the US. Outbreaks of porcine epidemic diarrhea (PED) continue to occur in the US, with over 6,000 PEDV-positive accessions reported from 29 states as of May 2014.

Contaminated livestock trailers represent a significant risk for movement of the PEDV between and within herds. One potential source of contamination for livestock trailers is the unloading dock at swine packing plants or harvest facilities[1]. Trailers from multiple swine sites of varying PEDV status come into contact with the unloading dock in short succession. If a trailer becomes contaminated with PEDV at the unloading dock, it may transport the virus to naïve pigs or a naïve swine site on its next load. Therefore, quick, practical, low cost methods of unloading dock sanitation need to be developed to prevent possible trailer contamination.

Recent research demonstrated that an accelerated hydrogen peroxide (AHP) disinfectant (Accel®, Ogena Solutions LLC, Stoney Creek, Ontario, Canada) inactivated porcine epidemic diarrhea virus (PEDV) at 20ºC after 30 minutes of contact time in the presence of significant amounts of fecal material. The concentrated form of the AHP disinfectant was efficacious at dilutions of 1:16 and 1:32. A follow-up study determined that the AHP disinfectant at 1:16 and 1:32 concentrations was also efficacious at inactivating PEDV under freezing conditions (-10ºC). These studies suggest that an AHP disinfectant may be a viable solution for PEDV sanitation protocols when organic matter is expected. However, the aforementioned research was completed on smooth metal surfaces with extended contact times (30, 40, and 60 minutes) which are not representative of the field conditions at a swine packing plant unloading dock.

Chlorine dioxide (CD), such as that found in the disinfectant ProKure V (ProKure Solutions, LLC, Phoenix, AZ), is a fast acting sanitizer, disinfectant, sterilizer, and decontaminant currently used to disinfect surfaces at hospitals, gyms, cruise ships, and research laboratories. CD is also used to sanitize pool water, drinking water, meat, and vegetables because it leaves no residue and produces no toxic or carcinogenic byproducts. CD's fast acting virucidal mechanism and approval for use in food contact areas make it a viable candidate for packing plant unloading dock sanitation where the lag time between trailers is short and strict regulations govern the products that can be applied.

OBJECTIVE

The objective of this study was to test the efficacy of an accelerated hydrogen peroxide (AHP) disinfectant and a Chlorine Dioxide (CD) disinfectant at inactivating PEDV in swine feces on concrete surfaces with a very short contact time under both warm (20ºC) and cold (-10ºC) conditions.

MATERIALS AND METHODS

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC) prior to the initiation of any experimental activity.

Source of animals and housing.

Forty, 3-week old barrows were sourced from a private commercial producer in Iowa. Pigs and their dams had no history of PEDV exposure. Four days prior to study initiation (study day -4), serum and rectal swabs from all pigs were submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) for diagnostic testing via ELISA and PCR to confirm that the pigs were PEDV, transmissible gastroenteritis virus and porcine reproductive and respiratory virus negative.

On arrival, each pig was identified with a unique plastic livestock ear tag and weighed. Following a 24 hour rest period and initial diagnostic screening, pigs were blocked by weight, stratified, and then randomly assigned to one of ten treatment groups (n=4) using a random number generator in Microsoft Excel®. Each group was housed in a different room within the Animal Biosafety Level 1 (ABSL-1) facility at the Iowa State University Veterinary Medical Research Institute (ISU VMRI). The four pigs in each room were housed in a raised tub and each pig was physically isolated from the other three pigs in the room with solid, transparent dividers to prevent the transmission of PEDV between replicates. The transparent dividers allowed the pigs to have visual and auditory, but not physical contact for the duration of the study. Each quadrant of the tub had dedicated feed and water sources. The pigs remained in individual housing from the time of inoculation to necropsy, or 7 days. Strips of plastic snow fence across the top of the tubs were secured with zip ties and bungie cords to create a ceiling and a large plastic sorting panel was placed diagonally across the tub to create a visual barrier (Figure 1).

Coupons

In this study, 40 concrete coupons were used (one for each pig). Concrete coupons were constructed using a mold designed by study personnel and QUIKRETE Gray Concrete Mix. Prior to mixing, the QUIKRETE Gray Concrete Mix was sifted to remove large aggregate pieces. Water was added to the sifted QUIKRETE Concrete mix until it had a peanut butter like consistency. This concrete was poured into a mold and allowed to cure for 3 days. After 3 days, the concrete coupons were removed from the mold and immersed in water for 5 days to complete the curing process. The concrete coupons were 3.81 cm thick at the edges and 20.32cm by 20.32 cm square. The inner portion of the coupon consists of a 7.62cm by 7.62cm square surrounded by a 1.91cm wide and 1.27 cm deep groove (Figure 2). This square and surrounding groove represents the non-slip “waffle” flooring present at swine packing plant dock and unloading areas.

Study Design

Ten treatment groups, including a Negative Control and Positive Control group were evaluated in this study. Two concentrations of AHP disinfectant (1:32 and 1:64), two concentrations of CD disinfectant (100 ppm and 50 ppm), and two temperatures (20°C and -10°C) were evaluated using a fixed level of fecal contamination (10 ml) and contact time (5 minutes). The Negative Control group was not sham disinfected, but the Positive Control group was sham disinfected with 30 ml of a sterile water solution that was 10% PG by volume. The Negative Control group used PEDV-negative feces. The Positive Control and all 8 disinfectant treatment groups used PEDV-positive feces. See Table 1 for a detailed description of the treatment groups evaluated.

Challenge Material

The PEDV positive feces used in this study were collected in a previous experiment in which 3-week old pigs were experimentally inoculated with PEDV. Feces were collected from clinically affected animals 7 days post-inoculation (DPI). Feces were collected in sterile 50 ml fecal cups and stored on ice until they could be frozen at -80^{0}C. On study day 0, fecal samples were thawed in a cool water bath and pooled into a single fecal homogenate to ensure uniform fecal characteristics for each replicate. A swab from this homogenate was submitted to the ISU VDL and tested to confirm the feces PEDV-positive status via by a nucleocapsid (N) gene-based quantitative real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). Diagnostic results confirmed that the homogenate was PEDV-positive with a cycle threshold (Ct) value of 19.4.

PEDV negative feces were collected from the negative control pigs in the study described above. Feces were collected, stored, thawed, and homogenized as previously described. A swab from the PEDV-negative feces homogenate was submitted to the ISU VDL and tested via PEDV real-time RT-PCR post-thaw to confirm the feces PEDV negative status prior to disinfectant treatment. Diagnostic results confirmed that the negative homogenate was PEDV-negative with a Ct value >35.

Contamination and Disinfection Procedures

All concrete coupons (20/40) used for treatment groups conducted at -10°C were placed into a standard chest freezer set to the coldest setting on study day -1, approximately 20 hours prior to inoculation, to ensure that the concrete was frozen upon fecal application. The remaining 20 coupons were stored at room temperature (20°C) in covered totes.

On study day 0, 10 ml of PEDV negative or PEDV positive feces, depending on treatment group, were applied to the concrete coupons in an even ≤ 4 mm layer using a disposable plastic adhesive spreaders and 4.5 inch wood craft sticks. Five (5) ml of feces were applied to the raised square in the center of the coupon using the adhesive spreader and the remaining 5 ml of feces were spread in the groove using the wood craft stick. A separate plastic adhesive spreader and craft stick were used for each coupon. Immediately after contamination (pre-treatment), environmental swabs were taken from each individual coupon using a commercial swab and transport system. The swabs were submitted to the ISU VDL and tested for the presence of viral ribonucleic acid (RNA) by PEDV Real-time RT-PCR.

All coupons, except the negative control, were allowed a 10 minute rest period prior to the application of the treatments described in Table 1. This rest period was designed simulate the feces present in swine packing plant unloading dock floors. These feces would accumulate as the pigs exit the trailer over approximately a 10 minute timeframe causing some feces to dry and adhere to the concrete surface while others remain wet.

Following the rest period, 30 ml (liquid volume) of the AHP disinfectant, CD disinfectant, or sham disinfectant were applied to each concrete coupon within the treatment group. Disinfectant application was staggered by coupon within each treatment group. This ensured that the entire sequence events from disinfectant application to pig inoculation took place as quickly as possible and preserved as close to a 5 minute contact time as possible.

AHP disinfectant was applied to contaminated coupons in the Warm 1:32 AHP, Warm 1:64 AHP, Cold 1:32 AHP, and Cold 1:64 AHP groups using a 1.5 gallon pump-up foamer. The AHP disinfectant solution was prepared using a 4.25% concentrate of the AHP disinfectant and tap water (Warm 1:32 AHP and Warm 1:64 AHP groups) or a 4.25% AHP concentrate, propylene glycol (PG) and tap water (Cold 1:32 AHP and Cold 1:64

AHP groups). A ratio of AHP concentrate to final solution of 1:32 was used for the Warm 1:32 AHP and Cold 1:32 AHP groups and a ratio of 1:64 was used for the Warm 1:64 AHP and Cold 1:64 AHP groups. The AHP disinfectant / PG mixture applied to the cold treatment groups was 10% PG by volume to prevent freezing.
CD disinfectant was applied to contaminated coupons in the Warm 100ppm CD, Warm 50ppm CD, Cold 100ppm CD, and Cold 50ppm CD treatment groups using a pump-up sprayer. The CD disinfectant solution was prepared using a 0.042g ProKure V Pouch charged in 1040 ml of tap water for 20 hours and then diluted with tap water (Warm 100ppm CD and Warm 50ppm CD groups) or tap water and PG (Cold 100ppm CD and Cold 50ppm CD groups) to reach the desired final concentration of either 100 ppm or 50 ppm CD. The CD disinfectant / PG solution applied to the Cold 100ppm CD and Cold 50ppm CD groups was 10% PG by volume to prevent freezing.
The positive control group was sham disinfected with 30 ml of a sterile water / PG solution. The sham disinfectant was 10% PG by volume and was applied using a separate pump-up sprayer to ensure that no residual disinfectant was present in the sprayer at the time of application. The negative control group was not sham disinfected.
After application of disinfectant, each coupon was allotted 5 minutes of contact time regardless of disinfectant treatment applied. Coupons in the Warm 1:32 AHP, Warm 1:64 AHP, Warm 100ppm CD and Warm 50ppm CD groups were held at room temperature (20°C) for the 5 minute contact time. Coupons from the Cold 1:32 AHP, Cold 1:64 AHP, Cold 100ppm CD, and Cold 50ppm CD groups were placed in a scientific freezer set to -10°C for the 5 minutes of contact time. Coupons in the negative control group were held at 20°C for 5 minutes after fecal contamination, but did not undergo disinfection.
An environmental swab was taken from each coupon post-treatment; after the 5 minute contact time. All post-treatment swabs were submitted to the ISU VDL and tested for the presence of PEDV RNA by PEDV Real-time RT-PCR.

Swine Bioassay
After the post-treatment swab was obtained, 10 ml of sterile 0.9% sodium chloride solution was applied to each coupon to facilitate fecal collection and aid with oral gavage. A toothbrush was used to re-suspend the feces / disinfectant solution mixture, creating a homogenate sample that served as the inoculum for the swine bioassay portion of the study. A new toothbrush was used for each coupon to prevent cross contamination between replicates. The feces / disinfectant homogenate was collected in a 20 ml syringe that was labeled with the coupon identifier and was matched to a single pig. A 3 ml aliquot of the inoculum was placed in a snap-cap tube and stored at -80°C in the event that further diagnostic testing was required.
The inoculum, feces / disinfectant solution / saline or feces / disinfectant solution / saline / PG, was administered to pigs via oral gastric gavage immediately after collection from the coupon to limit the additional contact time with the disinfectant. Each pig was inoculated with the contents of its designated concrete coupon with a 14 French rubber catheter. Personnel performing inoculation wore disposable TyVek® coveralls, a N95 respirator, arm-length disposable obstetrical sleeves and nitrile gloves. TyVeks and N95 respirators were changed between treatment groups; sooner if either became contaminated with inoculum during the gavage process. OB sleeves and nitrile gloves were discarded and changed between each pig.
Pigs were monitored daily for clinical signs consistent with PED. Rectal swabs were obtained from all pigs (40/40) 3 DPI and 7 DPI. These swabs were submitted to the ISU VDL and tested for the presence of PEDV

RNA by PEDV real-time RT-PCR. During rectal swab sampling, the same personnel protective equipment (PPE) procedures described for inoculation were used. Pigs were not removed from the tub during sampling. Pigs were euthanized on 7 DPI after the final rectal swab was obtained. All organ systems were grossly evaluated and any gross lesions or abnormal pathology was recorded. Fresh cecum and spiral colon contents and sections of fresh and 10% formalin-fixed mesenteric lymph nodes, ileum, and jejunum were collected from each pig. Fresh samples were placed in a cooler immediately upon collection and transferred to a -80°C for long term storage after necropsy of all 40 pigs was complete in the event further testing is required to confirm the bioassay results.

.

A pig was considered swine bioassay positive if its rectal swabs were PEDV-positive (Ct < 35) by PEDV real-time RT-PCR on both 3 and 7 DPI. A pig was considered swine bioassay negative if both rectal swabs (3 and 7 DPI) were PEDV-negative by PEDV Real-time RT-PCR. If results on days 3 and 7 DPI were different, further diagnostics on the fresh and fixed tissues were to be performed to confirm the status of the pig.

Statistical Analysis

The results of this study were inconclusive; therefore, no statistical analysis was performed.

RESULTS

Environmental swabs from all concrete coupons (36/36) to which PEDV-positive feces were applied (Positive Control, Warm 1:32 AHP, Warm 100ppm CD, Warm 1:64 AHP, Warm 50ppm CD, Cold 1:32 AHP, Cold 100ppm CD, Cold 1:64 AHP, and Cold 50ppm CD) tested positive for the presence of PEDV RNA via PEDV real-time RT-PCR before and after exposure to the designated disinfectant treatment. Pre-treatment and post-treatment environmental swabs from all concrete coupons (4/4, Negative Control) tested negative for the presence of PEDV RNA via PEDV real-time RT-PCR. Detailed results from each swab are summarized in Table 2.

Swine bioassay results were negative in 100% (4/4) of the pigs in the Negative Control, Warm 1:32 AHP, Warm 100ppm CD, Warm 1:64 AHP, Warm 50ppm CD, Cold 1:32 AHP, Cold 1:64 AHP, Cold 50ppm CD groups. One pig (1/4) in both the Cold 100ppm CD and Positive Control groups was suspect on 3 DPI. Using a cutoff off value (Ct >35), both of these samples were PEDV-negative. However, low amounts of genomic copies of PEDV were detected in both rectal swabs leading to the PEDV-suspect designation (Table 3). Three of the four pigs within the Positive Control Group were PEDV-negative on three days post-inoculation and all pigs (4/4) in the Positive Control group were PEDV-negative seven days post-inoculation. These results were unexpected as no disinfectant was applied to this group.

To validate the results received from the ante-mortem fecal swabs taken on 7 DPI, additional tests were conducted on cecum and colon contents collected post-mortem on 7 DPI. These samples were collected during necropsy and then placed in a -80°C freezer for storage. Cecum and colon contents were thawed in cool water and a sample was taken using a commercial swab and transport system. Swabs were placed in a snap cap tube and mixed with 1 ml of phosphate buffer solution (PBS) and submitted to Dr. Jianqiang Zhang for further testing. All samples were tested for the presence of PEDV RNA through 45 cycles of PEDV real-time RT-PCR (Table 4). One pig (1/4) in the Warm 1:32 AHP groups was PEDV-positive based on this test. All Positive Control pigs were PEDV suspect with Ct values ranging from 36.2 to 37.3, above the established negative cutoff of 35. Half (2/4) of the Negative Control pigs were PEDV suspect.

DISCUSSION

The positive control pigs failed to become positive through swine bioassay; therefore, the results from this study are inconclusive and cannot be used to determine the effectiveness of either the AHP or CD disinfectant on inactivating PEDV in swine feces on concrete surfaces.

PEDV real-time RT-PCR results on post-mortem cecum and colon contents (Table 4) from the 40 pigs enrolled in this study showed no definitive pattern. While all (4/4) Positive Control pigs were PEDV suspect from this test, these results are not enough to validate the study. Additionally, the results from this round of testing do not correlate with the initial testing performed on ante-mortem rectal swabs (Table 3). The ante-mortem rectal swabs indicated that 1 pig in the Cold 100ppm CD group and 1 pig in the positive control group were PEDV suspect on 3 DPI and no pigs (0/40) were PEDV-positive or PEDV-suspect on 7 DPI. However, the cecum and colon contents collected post-mortem indicated that 2/4 pigs in the Negative Control, 3/4 pigs in Warm 100ppm CD, 1/4 pigs in Warm 1:64 AHP, 1/4 pigs in the Cold 1:32 AHP, 3/4 pigs in the Cold 100ppm CD, and 4/4 pigs in the Positive Control groups were PEDV suspect on 7 DPI and 1/4 pigs in the Warm 1:32 AHP group was PEDV positive on 7 DPI. The high variability in these results may be contributed to the limitations of the current PEDV real-time RT-PCR used in this study. When PCR reaches the limit of detection, Ct values greater than the established cutoff value of 35, it is difficult to interpret and repeat the results. When very low amounts of viral RNA are present in the sample, repeatable and reliable results are rarely achieved and it is expected that if multiple PCR's were run on the same sample, several would test positive and several would test negative.

From the results described above, it is apparent that the PEDV present in the feces used in this study did not efficiently infect / propagate in inoculated pigs. To attempt to determine where the PEDV lost its infectivity, PEDV virus isolation was conducted on the following samples:

- Swab from PEDV-positive feces homogenate.
    - Swab was taken after thawing the frozen feces in a cool water bath, but prior to fecal application on concrete coupons.
- Pre-treatment swabs from all (4/4) Positive control concrete coupons.
    - Swab was taken immediately after 10 ml of feces was applied to concrete coupon
- Post-treatment swabs from all (4/4) Positive Control concrete coupons
    - Swab taken after coupon was sham-disinfected with a sterile water / PG solution
- Aliquot of inoculum given to each Positive Control pig

All samples, 9 swabs and 4 aliquots of inoculum, were confirmed PEDV-negative by virus isolation in both the 24 hour fast protocol and the 3 passages by 7 days protocol used at the ISU VDL. These results indicate that the PEDV may have been inactivated prior to its application to the concrete coupon. The inactivation may have occurred during collection, during long-term storage, or during the 10 minute rest period after it was applied to the concrete coupon.

The PEDV-positive feces used in this study were obtained from a previous study where 3-week old pigs were experimentally infected with PEDV. PEDV positive feces were collected during necropsy on 7 DPI from these pigs. After collection, feces were stored in a cooler until they were able to be placed in a -80°C freezer for long term storage. PEDV is susceptible to degradation at warmer temperatures.

The -80°C freezer where the feces were stored between trials malfunctioned during the storage period. ISU CVM staff in charge of the freezer noted that it was not holding temperature correctly for a period of time and substantial temperature fluctuations occurred until it was fixed. These temperature fluctuations may have caused the PEDV in the feces to endure additional freeze-thaw cycles, reducing the amount of viable virus in the

sample on the day of inoculation and resulting in the PEDV's inability to efficiently infect pigs during the bioassay portion of the trial.

The concrete coupons themselves may have further reduced the viability of the PEDV present in the swine feces. QUICKRETE contains calcium, magnesium, potassium and sodium salts in addition to crystalline silica sand, pulverized limestone, iron oxide pigments, lime and clay. When exposed to moisture, QUICKRETE become alkaline which can desiccate skin and other organisms that contact it. The concrete coupons were cured in a water bath for 5 days to counteract this drying effect, but were not treated with a chemical compound to further cure them. Once applied to the concrete coupon, some of the feces were absorbed into the concrete and the feces began to dry from exposure to air and concrete. It is possible, that a compound in the concrete coupon may have played a role in reducing the infectivity of the PEDV in the feces.

While no conclusions can be made about the efficacy of AHP and CD from the results of this study, it did provide further proof that a major limitation of real-time RT-PCR is that PCR cannot differentiate between infectious virus and fragments of RNA from inactivated virus. All post-treatment swabs, from the 36 coupons contaminated with PEDV positive feces and then subjected to one of eight AHP, CD, or sham disinfectant treatments, were positive for the presence of PEDV RNA by real-time RT-PCR post-treatment, but none of the coupons (0%) contained infectious virus as demonstrated by swine bioassay.

The PEDV positive feces used in this study were PEDV positive according to real-time RT-PCR, but were not infectious by swine bioassay; therefore, the efficacy of the AHP and CD disinfectant on inactivating PEDV in swine feces on concrete surfaces cannot be ascertained from the results of this study. Further research should be done to determine if either the AHP or CD disinfectant would be efficacious on PEDV on concrete. However, the results of this study did provide further evidence against using PCR as the sole method of determining if a sanitation protocol was effective as the feces used in this study were PEDV positive according to PCR, but the PEDV did not infect clinically healthy 3-week old barrows.

REFERENCES

1. Lowe J, Gauger P, Harmon K, Zhang J, Connor J, Yeske P, et al. Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. Emerg Infect Dis. 2014;20(5):872-874.

TABLES

Table 1: Summary of the treatment groups evaluated in this study and general study design.

Treatment Group	N	Contamination Material	Volume of Feces	Disinfectant Treatment	Contact Time	Temp	Route of Inoculation	Dose
Negative Control	4	PEDv-negative feces	10 ml	None	5 minutes	20^{0}C	Oral Gavage	Contents of tray + 10 ml saline
Positive Control	4	PEDv-positive feces	10 ml	Sham disinfectant (10% PG and Sterile Water)	5 minutes	-10^{0}C	Oral Gavage	Contents of tray + 10 ml saline
Warm 1:32 AHP	4	PEDv-positive feces	10 ml	AHP disinfectant 1:32	5 minutes	20^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Warm 100ppm CD	4	PEDv-positive feces	10 ml	CD disinfectant 100ppm	5 minutes	20^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Warm 1:64 AHP	4	PEDv-positive feces	10 ml	AHP disinfectant 1:64	5 minutes	20^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Warm 50ppm CD	4	PEDv-positive feces	10 ml	CD disinfectant 50ppm	5 minutes	20^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Cold 1:32 AHP	4	PEDv-positive feces	10 ml	AHP disinfectant 1:32 (10% PG by volume)	5 minutes	-10^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Cold 100ppm CD	4	PEDv-positive feces	10 ml	CD disinfectant 100ppm (10% PG by volume)	5 minutes	-10^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Cold 1:64 AHP	4	PEDv-positive feces	10 ml	AHP disinfectant 1:64 (10% PG by volume)	5 minutes	-10^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline
Cold 50ppm CD	4	PEDv-positive feces	10 ml	CD disinfectant 50ppm (10% PG by volume)	5 minutes	-10^{0}C	Oral Gavage	Contents of tray post-trt + 10 ml saline

Table 2. Summary of PEDV real-time RT-PCR results for the pre-treatment and post-treatment environmental swabs of forty concrete coupons

Treatment group*	Pre-treatment Ct Value (genomic copies/ml) †	Percentage positive or suspect for PEDV	Post-treatment Ct Value (genomic copies/ml) ‡	Percentage positive or suspect for PEDV
Negative Control	>35 (0)	0%	>35 (0)	0%
	>35 (0)		>35 (0)	
	>35 (0)		>35 (0)	
	>35 (0)		>35 (0)	
Warm 1:32 AHP	19.5 (71240900)	100%	21.9 (13257900)	100%
	19.2 (87344800)		19.3 (80097100)	
	18.8 (113296000)		20.3 (39795200)	
	19.2 (88505800)		21.1 (22726900)	
Warm 100ppm CD	18.0 (200348000)	100%	18.6 (13546200)	100%
	18.8 (117778000)		19.3 (81724100)	
	18.5 (14246500)\		19.5 (71439900)	
	19.0 (100688000)		19.7 (62481100)	
Warm 1:64 AHP	18.8 (118087000)	100%	19.3 (82520200)	100%
	19.1 (95001900)		20.1 (45543700)	
	18.6 (137338000)		19.8 (586134000	
	19.0 (101742000)		19.9 (55241300)	
Warm 50ppm CD	18.8 (119445000)	100%	19.3 (84320200)	100%
	19.2 (85812800)		19.0 (102876000)	
	18.3 (163502000)		18.1 (187070000)	
	19.0 (105007000)		19.8 (570469000	
Cold 1:32 AHP	19.4 (75420800)	100%	19.1 (97508000)	100%
	20.4 (37688900)		21.1 (24039600)	
	19.6 (66387100)		21.0 (25684400)	
	19.4 (75583900)		19.8 (56260000)	
Cold 100 ppm CD	19.8 (56408100)	100%	19.0 (104379000)	100%
	19.6 (69088400)		19.6 (65260800)	
	20.1 (48776600)		19.2 (90404100)	
	19.9 (54589100)		19.2 (90235500)	

Cold 1:64 AHP	19.6 (68440300)	100%	19. (87157800)	100%
	20.1 (47479900)		21.0 (25568900)	
	20.1 (48515600)		21.0 (25589100)	
	20.0 (52010000)		20.9 (26099600)	
Cold 50ppm CD	18.8 (11307800)	100%	18.6 (133630000)	100%
	19.3 (84049900)		18.4 (153479000)	
	20.0 (49059400)		19.3 (83157000)	
	18.9 (10604900)		18.2 (174449000)	
Positive Control	19.5 (69593300)	100%	19.4 (76630800)	100%
	20.2 (43503900)		18.1 (189160000)	
	19.2 (90905000)		19.4 (75055600)	
	19.9 (54613100)		19.1 (9423300)	

* Treatment groups are described in Table 1

† Pre-treatment swabs were taken immediately following fecal contamination of concrete coupons, prior to the 10 minute rest period and AHP or CD disinfectant treatment

‡ Negative Control: Post-treatment swabs were taken 5 minutes after pre-treatment swabs. Positive Control: post-treatment swabs were taken following sham disinfection and 5 minute contact time at -10°C. AHP and CD treatment groups: post-treatment swabs were taken after disinfectant application and 5 minute contact time at either 20°C or -10°C

Table 3: Summary of 3 DPI and 7DPI rectal swab PEDV real-time RT-PCR and swine bioassay results.

Treatment Group*	Day 3 Rectal Swab†; Percentage positive or suspect for PEDV RNA	Day 7 Rectal Swab‡; Percentage positive or suspect for PEDV RNA	Swine Bioassay Result♦; Percentage positive for PEDV RNA
Negative Control	0% (0/4)	0% (0/4)	0% (0/4)
Warm 1:32 AHP	0% (0/4)	0% (0/4)	0% (0/4)
Warm 100ppm CD	0% (0/4)	0% (0/4)	0% (0/4)
Warm 1:64 AHP	0% (0/4)	0% (0/4)	0% (0/4)
Warm 50ppm CD	0% (0/4)	0% (0/4)	0% (0/4)
Cold 1:32 AHP	0% (0/4)	0% (0/4)	0% (0/4)
Cold 100 ppm CD	25% (1 suspect/4)	0% (0/4)	0% (0/4)
Cold 1:64 AHP	0% (0/4)	0% (0/4)	0% (0/4)
Cold 50ppm CD	0% (0/4)	0% (0/4)	0% (0/4)
Positive Control	25% (1 suspect/4)	0% (0/4)	0% (0/4)

*Treatment groups are described in Table 1.

†Results from rectal swabs taken on study day 3 (3 DPI) and tested for PEDV by PEDV Real-time RT-PCR.

‡Results from rectal swabs taken on study day 7 (7 DPI) and tested for PEDV by PEDV Real-time RT-PCR.

♦Swine Bioassay was considered positive if rectal swabs on either day 3 or day 7 were PEDV positive by real time RT-PCR. Swine Bioassay was considered negative if rectal swabs on both day 3 and day 7 were PEDV negative by real-time RT-PCR.

Table 4: Summary of PEDV real-time RT-PCR results on post-mortem cecum and colon contents obtained from each pig during necropsy on 7 DPI to validate results obtained from ante-mortem fecal swabs obtained on 7 DPI

Treatment Group*	Cecum / Colon Contents Sample Ct Value	Genomic Copies / ml	PEDV Status
Negative Control	>45	0	Negative
	>45	0	Negative
	37.3	855.4	Suspect
	36.1	1927.2	Suspect
Warm 1:32 AHP	>45	0	Negative
	>45	0	Negative
	34.4	6097.2	Positive
	>45	0	Negative
Warm 100ppm CD	35.1	3685.7	Suspect
	36.2	1837.4	Suspect
	>45	0	Negative
	36.3	1725	Suspect
Warm 1:64 AHP	>45	0	Negative
	>45	0	Negative
	36.0	2043.6	Suspect
	>45	0	Negative
Warm 50ppm CD	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
Cold 1:32 AHP	35.7	2529.4	Suspect
	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
Cold 100 ppm CD	36.3	1706	Suspect
	>45	0	Negative
	36.2	1841.4	Suspect
	36.2	1810.8	Suspect

Cold 1:64 AHP	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
Cold 50ppm CD	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
	>45	0	Negative
Positive Control	36.2	1849.9	Suspect
	36.3	1679.6	Suspect
	36.2	1829.1	Suspect
	37.3	859.4	Suspect

*Treatment groups are described in Table 1.

FIGURES

Figure 1: Housing modifications made on study days 1 or 2 (post inoculation) to contain pigs within their respective quadrants of the elevated tub. A sorting panel was placed diagonally across the center of the tub creating a visual and physical barrier to prevent the pigs from using the feeders to escape their quadrant.

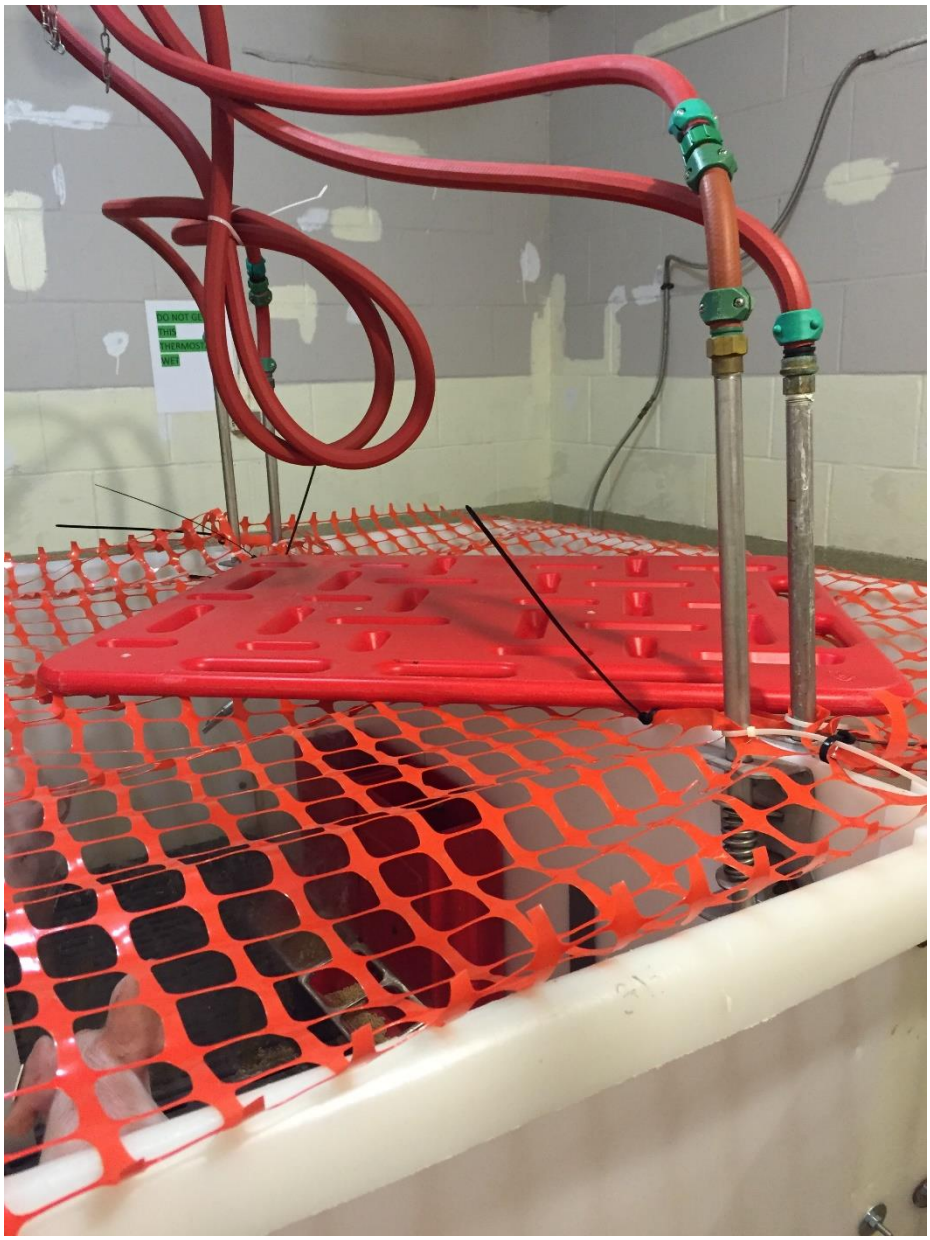

Figure 2: Concrete coupon measuring 20.32 cm by 20.32 cm with an inner 7.62 cm by 7.62 cm "waffle" surrounded by a 1.91 cm wide and 1.27 cm deep groove.