RESEARCH REPORT
SWINE HEALTH

Title: The effect of disinfection practices on RT-PCR detection of PEDv – NPB #14-151

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Industry Summary: Porcine epidemic diarrhea virus (PEDV) is causing significant damage to the United States pork industry. Rapid dissemination of this highly contagious virus across the nation has occurred, likely via contaminated transportation equipment. Cleaning and disinfection of trucks and trailers is widely practiced in the swine industry today and post-disinfection assessment is an important part of any protocol. Currently the detection of PEDV is limited to reverse transcription (RT)-PCR, a test which does not offer insight into the infectivity of detected PEDV but rather only identifies the presence of PEDV nucleic acid. Several classes of disinfectants render PEDV biologically inactive but do not disrupt the viral RNA. This situation results in samples testing positive when in fact the virus has been inactivated. In the present study we sought to test currently available disinfectants for their ability to both inactivate PEDV and disrupt the viral RNA so that PEDV cannot be detected by RT-PCR.

In the first objective, five commonly used classes of disinfectants were evaluated against PEDV in plastic petri dishes at varying concentrations, both in the presence and absence of swine feces to simulate field conditions. The testing was conducted under three different temperatures (37°C, 4°C, or -20°C) to mimic seasonal variations in ambient temperature. The disinfectants included in this study were a phenolic disinfectant, (One-stroke Environ); a quaternary ammonia compound (Roccal-D Plus); a chlorine compound, sodium hypochlorite (household bleach); an oxidizing agent, (Virkon S); and a quaternary ammonium/glutaraldehyde combination product (Synergize). Oxidizing agents and sodium hypochlorite are known to disrupt the RNA of other viruses thus three different dilutions of Virkon S (0.5%, 1% and 2%) and four different dilutions of sodium hypochlorite (0.17%, 0.52%, 1.03%, and 2.06%) were tested. All of the tested disinfectants were able to render PEDV non-infectious in cell culture. All the disinfectants except for 0.17% sodium hypochlorite produced significant reductions in the estimated number of PEDV copies on RT-PCR in all tested settings; however, none of the disinfectants were able to produce RT-PCR results that were completely negative across all replicates. Strong solutions of sodium hypochlorite (1.03% and 2.06%) and 0.5% oxidizing agent did produce several negative or nearly negative RT-PCR test results.

In the second objective, results from Objective 1 were used to select 2.06 % sodium hypochlorite and 0.5% oxidizing agent as disinfectants that showed potential to produce RT-PCR negative test results when used in the field. Aluminum coupons were pitted with 5% acetic acid to simulate a used livestock trailer. PEDV was applied to the aluminum coupons, allowed to dry, and then treated with either 2.06 % sodium hypochlorite or 0.5% oxidizing agent. The surface of the aluminum was swabbed and the swabs were tested with RT-PCR and used to inoculate

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th cells and naïve pigs. Neither of these two disinfectants was able to produce completely negative PCR results on pitted aluminum. While still PCR positive, the samples collected from the aluminum coupons were not infectious in cell culture or to naïve pigs.

All of the disinfectants tested in the present study were able to render PEDV non-infectious but few were able to disrupt the viral RNA to the point that PEDV could not be detected by RT-PCR. Because most PEDV strains do not grow in cell culture, the pork industry must rely upon RT-PCR for testing. Pork producers can expect to receive PCR positive test results even after proper disinfection with most commercially available disinfectants. Results of the present study indicate that oxidizing agents and sodium hypochlorite are most likely to produce RT-PCR results.

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Scientific Abstract: Currently the detection of PEDV is limited to RT-PCR and the present study sought to test disinfectants for their ability to both inactivate PEDV and disrupt the viral RNA so that PEDV became undetectable using RT-PCR. Five classes of disinfectants were evaluated in plastic petri dishes at varying concentrations, both in the presence and absence of swine feces. The testing was conducted under three different temperatures (37°C, 4°C, or -20°C). The disinfectants were a phenolic compound; a quaternary ammonia compound; sodium hypochlorite; an oxidizing agent; and a quaternary ammonium/glutaraldehyde combination. Three dilutions of the oxidizing agent (0.5%, 1% and 2%) and four dilutions of sodium hypochlorite (0.17%, 0.52%, 1.03%, and 2.06%) were tested. All of the tested disinfectants were able to render PEDV non-infectious in cell culture. All the disinfectants except for 0.17% sodium hypochlorite produced significant reductions in the estimated number of PEDV copies on RT-PCR in all tested settings; however, none of the disinfectants were able to produce RT-PCR results that were completely PEDV negative across all replicates. Strong solutions of sodium hypochlorite and 0.5% oxidizing agent did produce intermittent negative RT-PCR test results.

Secondly, aluminum coupons were pitted with 5% acetic acid to simulate a livestock trailer. PEDV was applied to the coupons, allowed to dry, and then treated with either 2.06 % sodium hypochlorite or 0.5% oxidizing agent. The surface of the aluminum was swabbed; the samples were tested with RT-PCR and used to inoculate both cells and naïve pigs. Neither of these two disinfectants was able to produce completely negative PCR results on pitted aluminum but these samples were not infectious in cell culture or to naïve pigs.

All of the disinfectants tested in the present study were able to render PEDV non-infectious but few were able to render PEDV undetectable with RT-PCR. Results of the present study indicate that oxidizing agents and sodium hypochlorite are most likely to produce negative RT-PCR results.

Introduction

The recent emergence of porcine epidemic diarrhea virus (PEDV) in the United States swine herd has had several detrimental impacts on the pork industry. Since the first reports of PEDV in Iowa in May, 2013 (Chen et al., 2013; Cima, 2013b), the highly contagious and deadly coronavirus (Figure 1) has rapidly spread across the nation. Common clinical signs include diarrhea and vomiting, which can lead to dehydration and electrolyte imbalance in infected animals. High mortality (70-100%) among neonates has led to significant economic losses in sow units (Cima, 2013a, 2013b). Before 2013, PEDV was seen only in Asian and European swine herds. Two PEDV strains, Korean DR13 and European CV777 strains have been grown in Vero cells, attenuated and were used as vaccines. However, CV777-vaccinated Chinese swine herds still developed PEDV outbreaks: China has lost more than 1 million pigs due to PEDV since 2010 (Sun et al., 2012). It is notable that the US PEDV strains are similar to one another and to the Chinese AH2012 strain (GenBank #KC210145) by phylogenetic analysis, sharing 99.8% nucleotide identity at the genomic level. Comparisons of the full 28 kb-genome sequences of five US PEDV strains from Colorado, Iowa and Minnesota suggests that US PEDV strains are likely of Chinese origin (Huang et al., 2013; Marthaler et al., 2013).

Transmission of PEDV occurs mainly through the oral-fecal route with acutely infected animals shedding large quantities of virus for several days post infection. The rapid emergence of highly similar strains across the United States indicates that transportation plays a major role in the spread of PEDV in this country. Contaminated transportation equipment has been linked to the spread of several other important swine diseases (PRRSV, *Salmonella, E. coli*) making trailer disinfection common among United States pork producers. The potential for devastating economic consequences of PEDV infection to the US pork industry requires efficient disinfection of PEDV on farm facilities and environment including trailers and trucks to prevent the spread of the disease.

PEDV is difficult to culture outside of an animal model; thus, RT-PCR assays are currently the only tests available to pork producers and swine veterinarians to detect PEDV. Because RT-PCR only detects the viral nucleic acid, a positive RT-PCR result only indicates detection of PEDV viral RNA, but does not mean viable and infectious virus is present. Because it is the only test available to them, pork producers are using RT-PCR to test trailers following disinfection to ensure that the equipment is free of PEDV before contact with naïve animals. The problem is that RT-PCR tends to underestimate disinfection efficacy compared to infectivity assays and producers are getting RT-PCR positive results when in fact the trailer has been disinfected and likely infective virus is not present. This drawback of RT-PCR has been recognized by many researchers for many pathogens (Pecson, Ackermann, & Kohn, 2011). This is because most disinfection agents act on the capsid that protects the viral RNA, and in many cases, inactivation has little or no effect on the viral RNA. For example, certain inactivation treatments, such as pasteurization, cause little or no damage to the viral genome (Pecson, Martin, & Kohn, 2009). Although the receptor ligands on a virion surface are damaged by the treatments, resulting in no infectivity, the virus particle may still be intact and viral RNA is protected within such a noninfectious viral particle, which can be detected by RT-PCR.

Previous studies have shown a wide variety of disinfectants effectively inactivate PEDV but we cannot detect this biological inactivation with RT-PCR. Presently there is a paucity of data examining disinfectant usage on PEDV RT-PCR results. While rapid progress is being made on viral culture methods, pork producers need practical solutions immediately to handle the discrepancy between RT-PCR and infectivity assays. In practical terms, pork producers must treat all RT-PCR positive trailers as infectious; the consequences of not doing so could be disastrous to their operation and the entire US swine industry. However, the cost associated with extra cleaning and disinfecting and additional down-time until a trailer tests negative is very expensive for pork producers. Therefore, we examined the effect of disinfectants on RT-PCR results and explored practical solutions to produce RT-PCR negative trailers after they have been contaminated with PEDV.

We conducted PEDV testing post-cleaning and disinfection trailers returning from harvest facilities from July through November 2013. Of those trailers tested, 10/150 (6.6%) have tested positive with RT-PCR following disinfection with a quaternary ammonium/glutaraldehyde combination product. Data from other pathogens indicated that accelerated peroxide-based compounds or sodium hypochlorite (bleach) would better disrupt the viral RNA and produce more meaningful RT-PCR results (Suarez et al., 2003). In a limited trial, we disinfected 25 trailers with potassium peroxymonosulfate instead of the quaternary ammonium/glutaraldehyde combination product and still had 4 (16%) RT-PCR positive trailers. Four previous manuscripts have demonstrated the ability of sodium hypochlorite or free chlorine to prevent RT-PCR detection in influenza A virus, hepatitis C virus, poliovirus, and rotavirus (Charrel, de Chesse, Decaudin, De Micco, & de Lamballerie, 2001; Ma, Straub, Pepper, & Gerba, 1994; Ojeh, Cusack, & Yolken, 1995; Suarez et al., 2003); thus, we expected the use of bleach to damage PEDV RNA such that it cannot be detected by RT-PCR. Additionally, bleach is commonly used in laboratory settings to control nucleic acid contamination.

Objectives:

The pork industry has been seeking methods to both inactivate PEDV and disrupt the viral RNA so that PEDV cannot be detected by RT-PCR. We hypothesized that selected commercially available disinfectants can sufficiently damage PEDV viral RNA resulting in negative RT-PCR test results. To that end the following objectives were proposed:

Objective 1: In vitro evaluation of disinfectants

The purpose of this objective is to evaluate five commonly used classes of disinfectants for their ability to both inactivate PEDV and disrupt the viral RNA so that PEDV cannot be detected by RT-PCR. This aim will include varying concentrations of most likely candidate compounds and will test the compounds' efficacy in the presence and absence of swine feces to simulate field conditions. The testing will be conducted under three different temperatures to mimic field conditions. Results of this aim will allow producers to understand the strengths and weaknesses of their disinfectant choices in regard to detection of PEDV.

Objective 2: Evaluation of a selected disinfectant on PEDV via bioassay

We will take the results of Aim 1 and select the disinfectant that is most likely to be successfully used in the field. We will then perform a bioassay with the selected protocol to confirm the effectiveness in a pig model. If Aim 1 is unable to identify a disinfectant that can make PEDV RNA undetectable, we will choose one disinfectant/protocol for Aim 2 that can reduce the estimated number of PEDV copies (RT-qPCR results) $\geq$3 log10 (99.9% reduction). Our preliminary data show that PEDV RNA is frequently detected from trailers that have been disinfected with various classes of disinfectants; however, application of certain disinfectants appears to decrease the frequency of positive RT-PCR results. Since procedures vary between pork producers, we seek to report applicable and useful results to producers. This aim will validate a practical and low-cost method to ensure meaningful RT-PCR results.

Materials & Methods

Objective 1

The disinfectants were initially tested for their effects on reducing RT-PCR detection of PEDV at three different temperatures (37°C, 4°C, or -20°C). Five commonly used disinfectants were evaluated for efficacy in inactivating PEDV and for their ability to disrupt PEDV RNA so that it cannot be detected by RT-PCR. The disinfectants to be included in this study were a phenolic disinfectant, (One-stroke Environ); a quaternary ammonia compound (Roccal-D Plus); a chlorine compound, sodium hypochlorite (household bleach); an oxidizing agent, (Virkon S); and a quaternary ammonium/glutaraldehyde combination product (Synergize). Oxidizing agents and sodium hypochlorite are known to disrupt the RNA of other viruses thus three different dilutions of Virkon S and four different dilutions of sodium hypochlorite were tested. Table 1 shows all the disinfectant dilutions, which were made using double distilled water.

The samples for the in vitro evaluation of disinfectants were generated using 147.8 cm^2 plastic petri dishes marked with 5 dots in a 2 ¾ inch square; three dishes were labeled for each substrate to be tested. One ml ($1x10^6$ $TCID_{50}$/ml) of PEDV (PC22A) was added to each petri dish and spread evenly to cover the surface of each dish using a cell spreader. DMEM with 7ug/ml trypsin, 1% pen/strep, and 0.3% tryptose phosphate broth was used as the negative control. The inoculum was allowed to dry completely in each petri dish in biosafety cabinets for 1 hour. Once dry, the dishes were incubated for 15 minutes at the selected temperature. After the incubation, 1 ml of each disinfectant was added to its respectively labeled petri dishes and spread evenly with a spreader. All disinfectants were allowed to dry in open petri dishes in biosafety cabinets for 1 hour. Once the disinfectants were dry, the lids were replaced on the petri dishes and the dishes were incubated for 15 minutes at the selected temperature. Double distilled water was used as the sham disinfectant for the positive and negative controls. A swab was pre-moistened in collection media (DMEM with 7ug/ml trypsin, 1% pen/strep, and 0.3% tryptose phosphate broth and 1ml DE neutralizing broth) prior to swabbing the dry petri dish. Each petri dish was swabbed in a "double W" pattern following the 5 marks on each petri dish and the swabs were placed into1 ml of collection media. The swabs and collection media were immediately mixed by vortexing, and incubated at room temperature (21ºC) for 30 minutes to allow the neutralizing broth time to neutralize any residual disinfectant.

One hundred microliters of each sample was used for RNA extraction using the Omega Mag-bind viral DNA/RNA kit and the MagMax Express Magnetic Particle Processor (Applied Biosystems, Foster, CA) according to the manufacturer's instructions. Following extraction, the RNA was subjected to real-time RT-PCR using a one-step multiplex QRT-PCR kit (Life Technologies Grand Island, NY. Path ID kit (cat#4442135).) in a 25 µl reaction mixture containing 12.5 µl 2X Multiplex buffer, 1.25 ul of the enzyme mix, 4.312 ul water, 1 ul of 5 uM probe, 0.469 ul of 40 uM forward primer, 0.469ul of 40uM reverse primer, and 5 µl extracted RNA. The primers and probes targeting the partial N gene were established in Dr. Wang's lab based on a previous publication (Kim et al., 2007) with modifications of forward primer based on the sequences of new US PEDV strains (PEDNFnew, 5'- CGCAAAGACTGAACCCACTAAC; PEDNR, 5'-TTGCCTCTGTTGTTACTTGGAGAT; and PEDprobe, FAM-TGYYACCAYYACCACGACTCCTGC-BHQ3). The reactions were performed on a QPCR system (Life Technologies 7500 Fast Real-Time PCR System) under the following thermocycling conditions: Stage 1–48 °C for 10 minutes, Stage 2–95 °C for 10 minutes, and Stage 3–45 cycles of 95 °C for 15 seconds and 60 °C for 40 seconds. Cycle threshold (Ct) values were calculated for each sample by setting the threshold at 5% of the positive control at cycle 40. Samples with a Ct of ≤40 were considered positive. A standard curve of the estimated TCID50 was generated for each RT-PCR run by making 10 fold dilutions of the positive control standard to estimate the TCID50 value of each positive sample. Estimated $TCID_{50}$ values were calculated based off standard curves generated with each RT-PCR run from the stock virus.

All samples were inoculated onto 96 well cell culture plates to determine if each disinfectant inactivated the virus. Vero cells (ATCC, CCL-81) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic cocktail (anti/anti). When confluent, the cells were washed with phosphate buffered saline (PBS) twice and then once with trypsin (0.25%). The trypsin wash was removed, fresh trypsin was added and the cells were incubated with trypsin for 15 minutes to help desensitize the cells to trypsin and prevent cell loss during inoculation. The trypsin was left in the flask and DMEM with 5% FBS and 1% anti/anti was added to inactive the remaining trypsin. Cells were plated onto 96 well plates to perform $TCID_{50}$ experiments.

The 96 well cell plates were washed twice with PBS, 50ul/well. Samples were ten-fold diluted in 96 well dummy plates containing DMEM with 7ug/ml trypsin, 1% penicillin-streptomycin solution, and 0.3% tryptose phosphate broth. The PBS wash was removed from the cells and 100ul of each dilution from the dummy plates were added to the 96 well cell culture plates. Cell plates were checked daily for cytopathic effects (CPE) and CPE was recorded on day three after inoculation. CPE was used to determine the $TCID_{50}$ value of each sample using the Reed & Muench method.

The above procedures were repeated with a 10% (v/v) fecal slurry made from PEDV negative pig feces and double distilled water. For the fecal samples, 1 ml of the 10% fecal slurry was added with the 1 ml of PEDv virus to the petri dishes. Due to the increased volume, the petri dishes with the fecal slurry were allowed to dry in biosafety cabinets for 90 minutes instead of 60 minutes.

All of the above procedures were performed at three separate temperatures (37°C, 4°C, or -20°C). Ultimately, each disinfectant was tested in triplicate at all three temperatures both with and without the 10% fecal slurry resulting in a total of 216 samples.

Table 1. Disinfectants and concentrations tested against tissue culture adapted PEDV strain PC22A in both cell culture media and 10% (v/v) swine feces slurry. Test was performed in triplicate resulting in 72 samples for each temperature tested. This procedure was replicated for each temperature (37ºC, 4ºC and -20ºC) for a grand total of 216 samples in Objective 1.

	Negative control	Positive Control	Quaternary ammonium (1.5:128)	Phenol (1:256)	Quaternary ammonium/glutaraldehyde combination (1:256)	Oxidizing agent (0.5%)	Oxidizing agent (1%)	Oxidizing agent (2%)	Sodium hypochlorite (0.17%)	Sodium hypochlorite (0.52%)	Sodium hypochlorite (1.03%)	Sodium hypochlorite (2.06%)
Cell culture Medium	3	3	3	3	3	3	3	3	3	3	3	3
Fecal Slurry	3	3	3	3	3	3	3	3	3	3	3	3

Objective 2

In this objective, the disinfectants that showed the highest likelihood for success in field applications were selected for further testing. Objective 1 results showed that 2.06 % sodium hypochlorite and 0.5% oxidizing agent (Virkon S) were the most effective at decreasing the detection of PEDv by RT-PCR; thus, were used in this part of the study. To mimic the surface of a livestock trailer, 10.4 cm x 10.4 cm x 0.6 cm aluminum coupons (bare plate aluminum 6061-T651) were used. Aluminum coupons were wiped with DNA AWAY (Molecular Bioproducts San Diego, CA), dried, rinsed with distilled water, soaked in a 5% acetic acid (distilled white vinegar) bath for 16 hours, rinsed with distilled water, and dried again. The aluminum coupons were then autoclaved at 121ºC for 15 minutes. Sterilized coupons were aseptically placed into sterile petri dishes in a biological safety cabinet. As in Objective 1, 1ml of stock PEDV ($1x10^6$ $TCID_{50}$/ml) was added to each aluminum coupon, spread over the entire coupon using a spreader, and allowed to dry for approximately 1 hour. Once dry the lids were replaced on the petri dishes and the dishes with aluminum coupons were incubated at 37°C for 15 minutes. After incubation the dishes were moved back to biosafety cabinets, the lids were removed, and 1 ml of respective disinfectant was added to each aluminum coupon. The disinfectant was spread over the entire surface of the coupon using a spreader and allowed to dry completely. Double distilled water was used as the 'disinfectant' for the positive and negative controls. Once dry the lids were replaced on the petri dishes and the dishes with aluminum coupons were incubated at 37°C for 15 minutes. A single swab was premoistened in

collection media and used to swab the entire aluminum coupon. The swab was put into 2 ml of collection media, mixed by vortexing, and a 150 ul aliquot was taken for RT-PCR. Samples were pooled by disinfectant and each pool was inoculated onto 96 well $TCID_{50}$ plates.

Bioassay

Twelve 3 week old PEDV negative and naïve pigs were divided into four challenge groups. Each pig was inoculated orally with 8.5ml of the pooled aluminum coupon sample of each treatement group. An additional 1 ml of each inoculum pool was saved for confirmatory $TCID_{50}$ and RT-PCR testing. Rectal swabs were collected daily for RT-PCR testing. Pigs were euthanized on day 10 post inoculation. Tissues were collected for histopathologic examination.

Results:

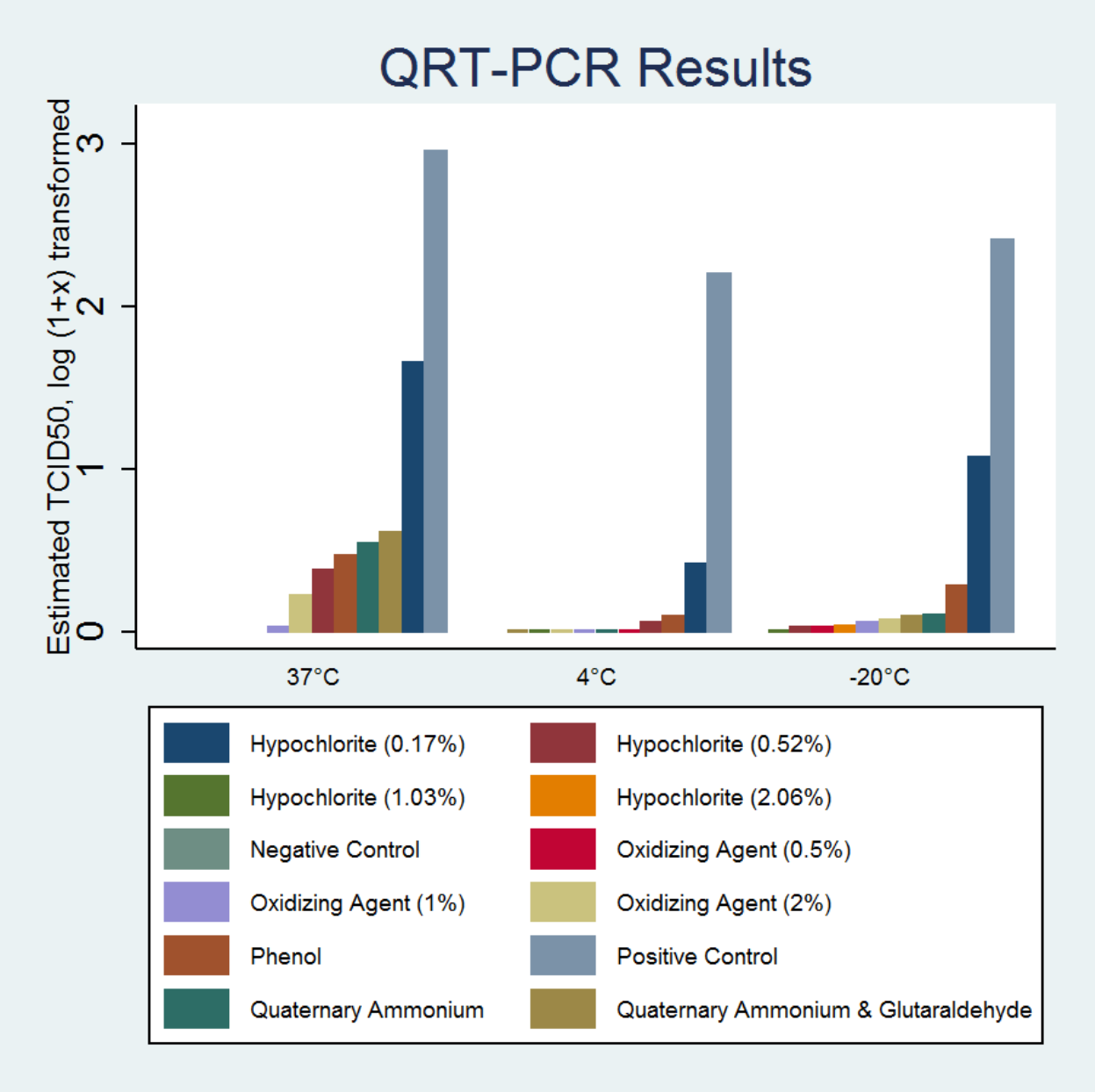
Objective 1

The QRT-PCR results are shown in Table 2 and Figures 1 and 2. All the disinfectants except for 0.17% sodium hypochlorite produced significant reductions in the estimated number of PEDV copies on QRT-PCR in all tested settings. None of the disinfectants were able to produce QRT-PCR results that were completely negative across all replicates; however, strong solutions of sodium hypochlorite (1.03% and 2.06%) and 0.5% oxidizing agent did produce several negative or nearly negative PCR test results (Table 2). All of the disinfectants were able to render PEDV non-infectious in cell culture.

Table 2: Mean QRT-PCR results (estimated $TCID_{50}$/ml) for the disinfectants tested against PEDV in cell culture medium or a 10%(v/v) fecal slurry.						
	37°C Cell Media	37°C Fecal Slurry	4°C Cell Media	4°C Fecal Slurry	-20°C Cell Media	-20°C Fecal Slurry
Negative Control	0	0	0	0	0	0
Quaternary Ammonium	3.137	0.184	0.017	0.003	0.292	0.114
Phenol	2.173	0.356	0.284	0.027	1.065	0.271
Quaternary Ammonium & Glutaraldehyde	4.368	0.045	0.006	0.115	0.262	0.846
Oxidizing Agent (0.5%)	0	0.032	0.028	0.001	0.077	0.264
Oxidizing Agent (1%)	0.086	0.040	0.015	0.011	0.149	0.102
Oxidizing Agent (2%)	0.718	0.079	0.014	0.008	0.185	0.089
Hypochlorite (0.17%)	68.745	51.160	1.996	87.449	11.692	21.663
Hypochlorite (0.52%)	3.292	0.005	0.145	0.019	0.070	0.763
Hypochlorite (1.03%)	0	0	0.012	0	0.015	0.016
Hypochlorite (2.06%)	0	0.002	0	0	0.096	0.207
Positive Control	942.925	228.643	171.853	54.612	255.528	137.103

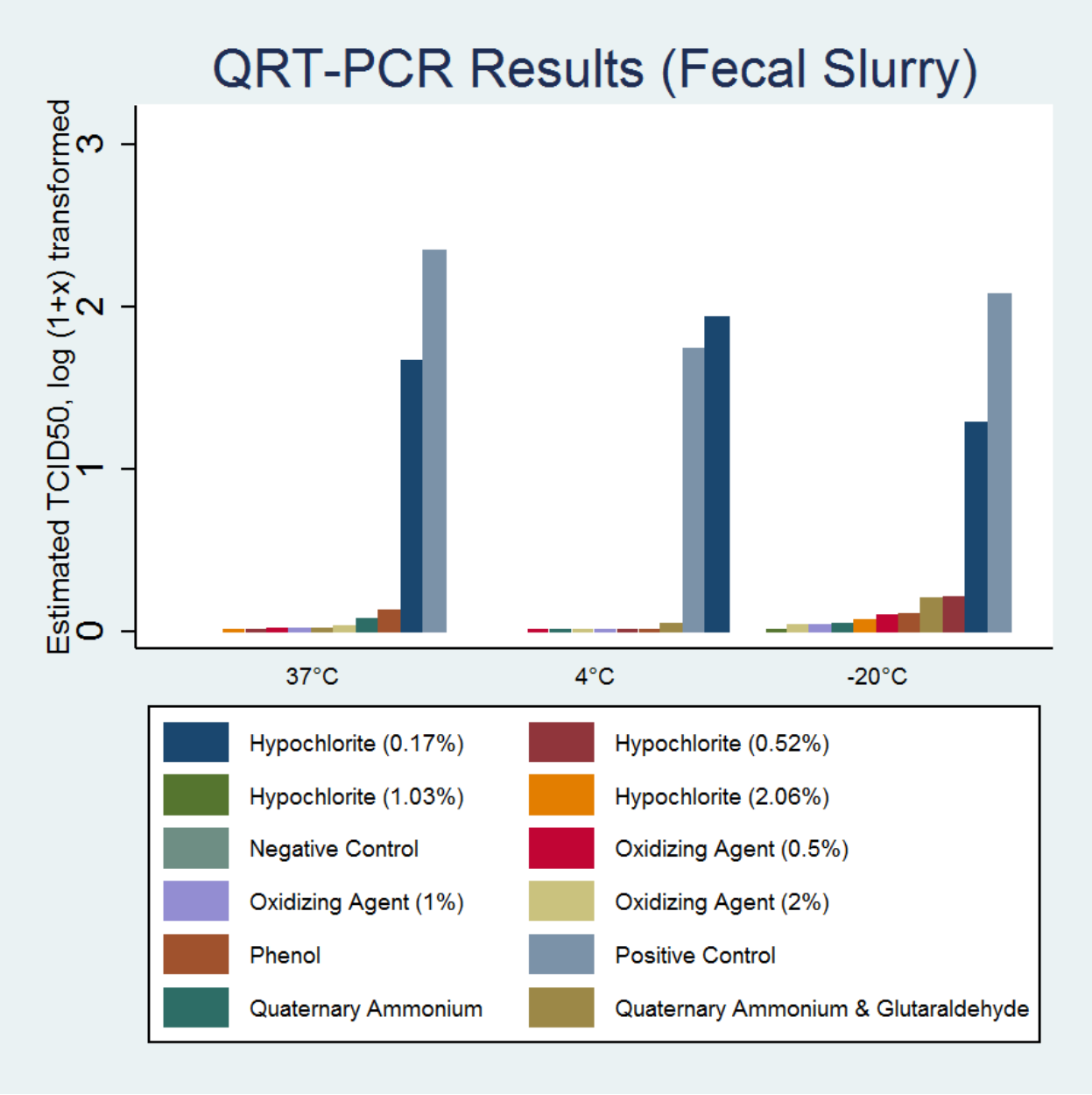
Cell Culture Media

Figure 1: Mean QRT-PCR results (estimated TCID50/ml that have been log (1+x) transformed) for the disinfectants tested against PEDV in cell culture medium.



Fecal Slurry

Figure 2: Mean QRT-PCR results (estimated TCID50/ml that have been log (1+x) transformed) for the disinfectants tested against PEDV in a 10% (v/v) fecal slurry.



Objective 2

Based on their performance in Objective 1, 2.06% sodium hypochlorite and 0.5% oxidizing agent were selected for use in Objective 2.

Table 3. Mean QRT-PCR results (estimated $TCID_{50}$/ml) for the disinfectants tested against PEDV in cell culture medium applied to pitted aluminum coupons.

	Mean Ct Value	Mean QRT-PCR results (estimated $TCID_{50}$/ml)
Positive Control	14.46	127.00
Oxidizing Agent (0.5%)	26.27	0.28
Hypochlorite (2.06%)	24.29	1.10
Negative Control	Negative	0

Infectivity

Viable PEDV was only recovered from the positive control samples. When inoculated onto Vero cells only the positive control samples yielded recovery of PEDV. In the bioassay, one of the positive control pigs tested PEDV positive on day 4 post inoculation and all three positive control pigs tested PEDV positive on 5 days post inoculation. PEDV was not detected in the rectal swabs from the negative control pigs nor the pigs challenged with the material collected from the swabbing of the aluminum coupons treated with 2.06% sodium hypochlorite and 0.5% oxidizing agent.

Discussion

All 10 of the disinfectants tested were able to render PEDV non-infectious but few were able to disrupt the viral RNA to the point that PEDV could not be detected by RT-PCR. Due to the fact that a strong solution of sodium hypochlorite (2.06%) and 0.5% oxidizing agent (Virkon S) did produce several negative or nearly negative PCR test results (Table 2), those two disinfectants were used for Objective 2. When applied to pitted aluminum coupons to simulate a livestock trailer, neither of these two disinfectants were able to produce completely negative PCR results. The recorded PCR results were near the limit of detection and significantly less than the positive control but nonetheless still detectable. The samples collected from the aluminum coupons were not infectious in cell culture or in naïve pigs.

Because most PEDV strains do not grow in cell culture, the pork industry must rely upon RT-PCR for testing. Pork producers can expect to receive PCR positive test results even after proper disinfection with most commercially available disinfectants. Results of the present study indicate that oxidizing agents and sodium hypochlorite are most likely to produce RT-PCR results. It should be noted that 2.06% sodium hypochlorite is a very strong concentration of bleach that is consider a serious hazard to human health. Contact with bare skin and can result in chemical burns and inhalation of sodium hypochlorite irritates the respiratory tract and can cause in pulmonary edema.