Methods for inactivating PEDV in Hog Trailers

Paul Thomas,[*,‡] DVM; **L. A. Karriker**,[*,†] DVM, MS, DACVPM; **A. Ramirez**,[†] DVM, M.P.H., PhD, DACVPM; **J. Zhang**,[†] M.D., MS, PhD; **J. S. Ellingson**,[*,‡] DVM, MS; **D. J. Holtkamp**,[†] DVM, MS
[*] Swine Medicine Education Center, College of Veterinary Medicine, Iowa State University, Ames, Iowa
[†] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa
[‡] AMVC Management Services, Audubon, Iowa

Introduction

Porcine epidemic diarrhea (PED) was first described in England in 1971 in growing pigs,[1] and the causative agent, porcine epidemic diarrhea virus (PEDV), was identified in 1978.[2,3] The virus spread to the rest of Europe where it caused outbreaks of diarrhea and significant losses throughout the 1970s and 1980s.[4,5] PEDV is considered endemic to Europe today, but does not cause widespread significant disease. In parts of Asia outbreaks were recognized first in 1982 and have continued to occur since that time.[4,5] In May of 2013 PEDV was identified in swine for the first time in the United States. The virus has caused severe diarrhea in sows and piglets, with near 100% mortality in piglets across a wide geographical area of the United States.[6] Genetic analysis of PEDV isolates from affected farms in the United States found the virus to be 99% genetically similar to isolates from China,[7,8,9] but efforts to determine the source of entry to the United States have been unsuccessful.

Although the original mode of entry of PEDV into the United States remains unknown, contaminated livestock trailers certainly represent a significant risk for movement of the virus between and within herds.[1,2] This is true of other swine diseases as well including porcine reproductive and respiratory syndrome virus (PRRSV)[1,3] and transmissible gastroenteritis virus (TGEV). Historically, this disease risk has been effectively mitigated in some cases with the use of trailer washing, disinfection protocols, and thermo-assisted drying and decontamination (TADD) systems.[1,4] Considering the effectiveness of a wash, disinfect, and TADD program to control these other diseases and the structural similarity of PEDV to TGEV, this program should be an efficacious means of inactivating PEDV in contaminated livestock trailers.

This paper summarizes four studies that evaluated individual aspects of trailer sanitation programs including TADD and multiple disinfectants alone, as well as several protocols that included washing, disinfection, and TADD.

Experimental Design

Four separate studies were conducted from September 2013 through July 2014 to evaluate different aspects of commonly utilized trailer sanitation protocols. Study 1 evaluated time and temperature combinations required to inactivate PEDV in feces. Study 2 evaluated the efficacy of Stalosan® F disinfectant powder to inactivate PEDV in feces when applied to a contaminated hog trailer. Study 3 evaluated the efficacy of Accel® disinfectant to inactivate PEDV in feces. Studies 1 to 3 were conducted without attempted removal of feces in order to evaluate each intervention's performance in the absence of trailer power washing. Study 4 evaluated multiple trailer sanitation protocols that included wash, disinfection, and TADD steps.

Each study consisted of a treatment step in which the intervention of interest was applied to an aluminum tray contaminated with PEDV-positive feces, and a bioassay step to determine if infectious virus was present and thereby evaluate the efficacy of the intervention of interest. In every study each individual tray was matched to an individual pig.

Study 1

Eight groups representing different combinations of time and temperature were evaluated. Five mL of undiluted PEDV-positive feces (or negative feces for the negative control group) was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Following treatment as outlined in Table 1, the feces was diluted with 10 mL of saline, re-collected from the tray, and passed via gastric tube into PEDV-naïve 4-week old pigs. These pigs served as a bioassay to detect the presence of infectious PEDV. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR to determine bioassay status. The individual pig was the experimental unit and each treatment group contained 4 replicates.

Table 1. Description of treatment groups and bioassay outcomes for Study 1.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Negative control	No treatment, pigs received a gavage of PEDV-negative feces	0% (0/4) [a]
Positive control	No treatment, pigs received a gavage of PEDV-positive feces	100% (4/4) [b]
71C-10M	PEDV-positive heated to 71° C (160° F) in an incubator and held at this temperature for 10 minutes.	0% (0/4) [a]
63C-10M	PEDV-positive feces heated to 63° C (145° F) in an incubator and held at this temperature for 10 minutes	25% (1/4) [a, b]
54C-10M	PEDV-positive feces heated to 54° C (130° F) in an incubator and held at this temperature for 10 minutes.	25% (1/4) [a, b]
38C-12H	PEDV-positive feces heated to 38° C (100° F) in an incubator and held at this temperature for 12 hours	50% (2/4) [a, b]
20C-24H	PEDV-positive feces left at 20° C (room temperature) for 24 hours	25% (1/4) [a, b]
20C-7D	PEDV-positive feces left at 20° C (room temperature) for 7 days	0% (0/4) [a]

Groups with different superscripts indicate statistically significant differences ($P \leq 0.05$).

Study 2

A Stalosan® F treatment group and positive and negative control groups were evaluated. Five mL of undiluted PEDV-positive feces (or negative feces for the negative control group) was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Trays from the negative and positive control groups were covered with a sealed lid to prevent contact with Stalosan® F disinfectant powder during treatment. Aluminum trays were then placed in various locations on the floor and walls within a commercial hog trailer. Stalosan® F disinfectant powder was then blown throughout the trailer with an electric leaf blower at a rate of 81 grams per meter^2 and allowed to contact the trays in such a way as determined by the natural movement of the powder through the trailer. During the one hour period of contact time, the trays were removed from the trailer and placed indoors at room temperature (20°C). Following treatment the feces were diluted with 10 mL of saline, re-collected from the tray, and 4 mL was removed for other use. The remaining mixture (~6-8 mL) was passed via gastric tube into PEDV-naïve 4-week old pigs. The individual pig was the experimental unit and a single pig corresponded to a single tray. Each treatment group contained 8 replicates, with each pig being an experimental unit. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR to determine bioassay status.

Table 2. Description of treatment groups and bioassay outcomes for Study 2.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Negative Control	PEDV-negative feces, no Stalosan® F contact	0% (0/8) [a]
Positive Control	PEDV-positive feces, no Stalosan® F contact	100% (8/8) [b]
Stalosan® F Treatment	PEDV-postive feces, one hour of Stalosan® F contact	100% (8/8) [b]

Groups with different superscripts indicate statistically significant differences ($P \leq 0.05$).

Study 3

Six groups representing different combinations of fecal contamination (light vs. heavy) and disinfectant concentration (1:16 vs. 1:32) were evaluated. Five or 10 mL of undiluted PEDV-positive feces (or negative feces for the negative control group) was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Following treatment with Accel® disinfectant as outlined in Table 1, the feces were diluted with 10 mL of saline, re-collected from the tray, and passed via gastric tube into PEDV-naïve 4-week old pigs. These pigs served as a bioassay to detect the presence of infectious PEDV. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR to determine bioassay status. The individual pig was the experimental unit, and each treatment group contained 4 replicates.

In addition to these treatment groups, a transmission control group was created to evaluate the validity of our individual pig-housing environment used in every study. Pigs were housed individually with 4 pigs housed in an elevated tub that was partitioned into four individual pens (Figure 1). Partitions were sealed so that no fecal material could pass between pens, and pigs had visual contact, but absolutely no nose-to-nose contact. The transmission control group consisted of one PEDV-positive pig (challenge was identical to the positive control group) and three PEDV-negative pigs (challenge identical to negative control group). Pigs were cared for, handled, evaluated, and tested identically to all other groups to determine if our methods could result in transmission of PEDV from one infected pig to others in the group. Pigs were monitored for clinical signs consistent with PED, and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR to determine bioassay status.

Figure 1. Elevated tubs used to house pigs for duration of the study. One tub was located in each room and each tub was split into quarters with one pig per quarter. Design of the tub prevented contact between pigs and movement of feces or other waste between tub quarters.

Table 3. Description of treatment groups and bioassay outcomes for Study 3.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Negative control	No treatment, pigs received a gavage of PEDV-negative feces	0% (0/3) a
Positive control	No treatment, pigs received a gavage of PEDV-positive feces	100% (4/4) b
5mL-1:16	A 1:16 concentration of Accel® disinfectant was applied to 5 ml of PEDV-positive feces for 30 minutes	0% (0/4) a
10mL-1:16	A 1:16 concentration of Accel® disinfectant was applied to 10 ml of PEDV-positive feces for 30 minutes	0% (0/4) a
5mL-1:32	A 1:32 concentration of Accel® disinfectant was applied to 5 ml of PEDV-positive feces for 30 minutes	0% (0/4) a
10mL-1:32	A 1:32 concentration of Accel® disinfectant was applied to 10 ml of PEDV-positive feces for 30 minutes	0% (0/4) a
Transmission control	¼ pigs in the group was gavaged with PEDV-positive feces, ¾ were gavaged with PEDV-negative feces	25% (1/4)

Groups with different superscripts indicate statistically significant differences ($P \leq 0.05$).
Results from the transmission control group were not included in statistical analysis.

Study 4

Nine groups representing power washing with detergent followed by different combinations of Synergize® disinfectant contact time and various TADD protocols were evaluated. In each protocol, 10 mL of undiluted PEDV-positive feces (or negative feces for the negative control group) was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Trays were then power washed with the aid of a detergent and underwent further sanitation with a disinfection step and potential TADD step. Following each of these treatments as outlined in Table 1, the remaining material in the tray was diluted with 10 mL of saline, re-collected from the tray, and passed via gastric tube into PEDV-naïve 4-week old pigs. These pigs served as a bioassay to detect the presence of infectious PEDV. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR to determine bioassay status. The individual pig was the experimental unit and each treatment group contained 4 replicates.

Table 4. Description of treatment groups and bioassay outcomes for Study 4.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Negative control	No treatment, pigs received a gavage of PEDV-negative feces	0% (0/4) [a]
Positive control	No treatment, pigs received a gavage of PEDV-positive feces	100% (4/4) [b]
WD-68C-10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 68° C (155° F) in an incubator and held at this temperature for 10 minutes	0% (0/4) [a]
WD-66C-10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 66° C (150° F) in an incubator and held at this temperature for 10 minutes	0% (0/4) [a]
WD-60C-20	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 60° C (140° F) in an incubator and held at this temperature for 20 minutes	0% (0/4) [a]
WD-49C-20	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, heated to 49° C (120° F) in an incubator and held at this temperature for 20 minutes	0% (0/4) [a]
WD-20C-12	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes, left at 20° C (room temperature) for 12 hours	0% (0/4) [a]
WD60	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 60 minutes	0% (0/4) [a]
WD10	PEDV-positive feces power washed with detergent, application of 1:256 concentration of Synergize® disinfectant for 10 minutes	0% (0/4) [a]

Groups with different superscripts indicate statistically significant differences ($P \leq 0.05$).

Results

PEDV swine bioassay results were analyzed using Fisher's Exact test (SAS® Enterprise Guide 5.1, Cary, NC, USA) for pairwise comparisons of all groups within a study. No between study comparisons were made or implied. In Study 1, the 71C-10M and 20C-7D groups were each found to be 100% effective at inactivating PEDV and significantly different than the positive control group (P=0.0286). No other group comparisons were found to be significantly different from one another using P<0.05 as a cutoff for significance. In Study 2, the proportion of PEDV-positive bioassays in the Stalosan® F treatment group was found to be no different than the Positive Control. In Study 3, all treatment groups (5mL-1:16, 5mL-1:32, 10mL-1:16, and 10mL-1:32) were 100% effective at inactivating PEDV to the point of preventing infection (P=0.0286). Within the transmission control group, the one positive pig did not transmit PEDV to the 3 negative pigs during the duration of the trial. In Study 4, all treatment groups (WD-68C-10, WD-66C-10, WD-60C-20, WD-49C-20, WD-20C-12, WD60, WD10) were 100% effective at inactivating PEDV (P=0.0286). Results are summarized with more detail in Tables 1 to 4.

Discussion

These results suggest that it may be possible to inactivate PEDV in the presence of feces by heating trailers to 71°C for 10 minutes or by maintaining them at room temperature (20°C) for at least 7 days. No other combinations of time and temperature alone were shown to be effective at inactivating PEDV.

Additionally, it appears that Accel® disinfectant was effective at inactivating PEDV in the presence of both heavy (groups 10mL-1:16 and 10mL-1:32) and light (groups 5mL-1:16 and 5mL-1:32) fecal contamination. Accel® was also found to be effective at half the recommended rate (groups 5mL-1:32 and 10mL-1:32).

In contrast, the other disinfectant-only study (Study 2) demonstrated that Stalosan® F disinfectant powder alone did not inactivate PEDV in feces. This demonstrates the importance of evaluating proper disinfection choices for different applications. Disinfectants vary widely not only in their spectrum against pathogens, but in their physical properties as well. These properties include characteristics like liquid vs. powder and different foaming qualities. While the spectrum of activity is very important, these other properties are also important because they affect the application of the disinfectant and its ability to remain in contact with surfaces.

Study 4 demonstrates the value of a complete trailer sanitation protocol that includes a wash step, disinfection step, and a final heating step. In that study, all treatment groups were effective at inactivating PEDV to the point of preventing infection in 3-week old pigs. Furthermore, it is important to note that temperatures that were found to be ineffective under the conditions of Study 1 (63°C for 10 minutes, 54°C for 10 minutes, 38°C for 12 hours, 20°C for 24 hours) were effective following a wash and disinfection step.

The investigators do not propose that either a TADD-only or disinfectant-only approach to trailer sanitation is a preferred alternative to thoroughly washing, disinfecting, and drying trailers. Indeed, Study 4 demonstrated the value of including washing, disinfecting, and heating in a trailer sanitation protocol. Rather, this work demonstrates the value of possible alternatives when proper washing and disinfection cannot be accomplished as a means to reduce the risk of transmitting PEDV between groups of animals. This work also demonstrates that assumptions about temperature and time targets for TADD systems that are valid following washing and disinfection steps are not valid in the absence of those steps.

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