

# Methods for Inactivating PEDV in Hog Trailers

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### Introduction

Porcine epidemic diarrhea (PED) was first described in England in 1971 in growing pigs and the causative agent, porcine epidemic diarrhea virus (PEDV), was identified in 1978. The virus spread to the rest of Europe where it caused outbreaks of diarrhea and significant losses throughout the 1970s and 1980s. 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. 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. Genetic analysis of PEDV isolates from affected farms in the US found the virus to be 99% genetically similar to isolates from China, 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 US remains unknown, contaminated livestock trailers certainly represent a significant risk for movement of the virus between and within herds. This is true of other swine diseases as well including porcine reproductive and respiratory syndrome virus (PRRSV) 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. 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 several protocols that include washing, disinfection and TADD.

### Materials and Methods

This is a summary of four studies conducted from September 2013 through July 2014 to evaluate the efficacy of different aspects of commonly used trailer sanitation protocols for inactivating porcine epidemic diarrhea virus (PEDV). The same experimental model was used in all four studies. A fixed volume (5 or 10 ml) of undiluted PEDV-positive feces from pigs intentionally infected with PEDV (or negative feces for the negative control group) was spread evenly on the floor of a 6 by 6 inch aluminum tray with 1 inch sides. The trays were made to replicate the floor of a livestock trailer. Following treatment as outlined in Tables 1 to 4 for each study, the feces was re-collected from the trays, and given to PEDV-naïve 4-week old pigs via gastric tube. These pigs served as a bioassay to detect the presence of infectious PEDV remaining in the treated feces. Fecal swabs were collected from the pigs on days 3 and 7 post-challenge. The fecal swabs were tested for the presence of PEDV by PCR to determine if the pigs were infected by the material in each tray. Each treatment group contained 4 replicates (4 trays and 4 pigs; 1 tray per pig). All 4 pigs in a treatment group were housed separately in raised tubs but in the same room.

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## Results

**Study 1** evaluated time and temperature combinations required to inactivate PEDV in feces. Treatment groups evaluated and the bioassay results are in Table 1.

**Table 1.** Description of treatment groups and bioassay outcomes for study 1.

<b>Treatment group</b>	<b>Description of treatment</b>	<b>Percentage of PEDV positives (out of 4)</b>
Neg control	PEDV-negative feces, no treatment	0% (0/4)
Pos control	PEDV-positive feces, no treatment	100% (4/4)
71C-10M	PEDV-positive feces heated to 71° C (160° F) and held at this temperature for 10 minutes.	0% (0/4)
63C-10M	PEDV-positive feces heated to 63° C (145° F) and held at this temperature for 10 minutes	25% (1/4)
54C-10M	PEDV-positive feces heated to 54° C (130° F) and held at this temperature for 10 minutes.	25% (1/4)
38C-12H	PEDV-positive feces heated to 38° C (100° F) and held at this temperature for 12 hours	50% (2/4)
20C-24H	PEDV-positive feces left at 20° C (room temperature) for 24 hours	25% (1/4)
20C-7D	PEDV-positive feces left at 20° C (room temperature) for 7 days	0% (0/4)

The 71C-10M and 20C-7D treatment groups were each found to be 100% effective at inactivating PEDV. No other groups were found to be effective.

**Study 2** evaluated the efficacy of Stalosan<sup>®</sup> F disinfectant powder to inactivate PEDV in feces. Treatment groups evaluated and the bioassay results are in Table 2.

**Table 2.** Description of treatment groups and bioassay outcomes for study 2.

<b>Treatment group</b>	<b>Description of Treatment</b>	<b>Percentage of PEDV positives (out of 8)</b>
Neg control	PEDV-negative feces, no treatment	0% (0/8)
Pos control	PEDV-positive feces, no treatment	100% (8/8)
Stalosan <sup>®</sup> F	PEDV-positive feces, Stalosan <sup>®</sup> F with one hour of contact time at 20° C (room temperature)	100% (8/8)

The Stalosan<sup>®</sup> F treatment was not effective at inactivating PEDV.

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**Study 3** evaluated the efficacy of Accel<sup>®</sup> disinfectant to inactivate PEDV in feces. Treatment groups evaluated and the bioassay results are in Table 3.

**Table 3.** Description of treatment groups and bioassay outcomes for study 3.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Neg control	PEDV-negative feces, no treatment	0% (0/3)
Pos control	PEDV-positive feces, no treatment	100% (4/4)
5mL-1:16	A 1:16 concentration of Accel <sup>®</sup> disinfectant applied to 5 ml of PEDV-positive feces for 30 minutes at 20° C (room temperature)	0% (0/4)
10mL-1:16	A 1:16 concentration of Accel <sup>®</sup> disinfectant applied to 10 ml of PEDV-positive feces for 30 minutes at 20° C (room temperature)	0% (0/4)
5mL-1:32	A 1:32 concentration of Accel <sup>®</sup> disinfectant applied to 5 ml of PEDV-positive feces for 30 minutes at 20° C (room temperature)	0% (0/4)
10mL-1:32	A 1:32 concentration of Accel <sup>®</sup> disinfectant applied to 10 ml of PEDV-positive feces for 30 minutes at 20° C (room temperature)	0% (0/4)
Transmission control	1 of 4 pigs in the group was gavaged with PEDV-positive feces, 3 of 4 were gavaged with PEDV-negative feces	25% (1/4)

All Accel<sup>®</sup> treatment groups (5mL-1:16, 5mL-1:32, 10mL-1:16, and 10mL-1:32) were 100% effective at inactivating PEDV. The transmission control group was included to validate that the animal housing and handling protocols used for the bioassay were preventing transmission of virus from

one pig to another within the same treatment group. Within the transmission control group, the one positive pig did not transmit PEDV to the 3 negative pigs during the duration of the trial.

**Study 4** evaluated multiple trailer sanitation protocols that included wash, disinfection, and TADD steps. Treatment groups evaluated and the bioassay results are in Table 4.

**Table 4.** Description of treatment groups and bioassay outcomes for study 4.

Treatment group	Description of treatment	Percentage of PEDV positives (out of 4)
Neg control	PEDV-negative feces, no treatment	0% (0/4)
Pos control	PEDV-positive feces, no treatment	100% (4/4)
WD-68C-10	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes, heated to 68° C (155° F) and held at this temperature for 10 minutes	0% (0/4)
WD-66C-10	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes, heated to 66° C (150° F) and held at this temperature for 10 minutes	0% (0/4)
WD-60C-20	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes, heated to 60° C (140° F) and held at this temperature for 20 minutes	0% (0/4)
WD-49C-20	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes, heated to 49° C (120° F) and held at this temperature for 20 minutes	0% (0/4)
WD-20C-12	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes, left at 20° C (room temperature) for 12 hours	0% (0/4)
WD60	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 60 minutes	0% (0/4)
WD10	PEDV-positive feces pressure washed with detergent, Synergize <sup>®</sup> disinfectant at 1:256 concentration for 10 minutes	0% (0/4)

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.

### Discussion and Implications

- PEDV in the presence of feces was inactivated by heating to 71°C for 10 minutes or by maintaining room temperature (20°C) for at least 7 days. No other combinations of time and temperature evaluated were shown to be effective at inactivating PEDV.
- Stalosan<sup>®</sup> F disinfectant powder alone with one hour of contact time at room temperature did not inactivate PEDV in feces.
- Accel<sup>®</sup> disinfectant was effective at inactivating PEDV in the presence of both heavy and light fecal contamination with 30 minutes of contact time at room temperature. Accel<sup>®</sup> was found to be effective at both concentrations evaluated (1:16 and 1:32).
- The results for studies 1 through 3 demonstrate the importance of evaluating proper disinfection choices for different applications. Disinfectants vary widely not only in their spectrum against pathogens and their physical properties. Properties include liquid versus 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.
- Complete trailer sanitation protocols that included a wash with detergent, disinfection with Synergize<sup>®</sup> at a concentration of 1:256, and heating were effective at inactivating PEDV.
- Synergize<sup>®</sup> alone with 10 or 60 minutes of contact time following a wash with detergent and disinfection was effective with or without heating after disinfection.

The sanitation procedures evaluated in studies 1, 2 and 3 were conducted without removal of feces. The investigators do not propose that TADD-only or disinfectant-only approaches to trailer sanitation are preferred alternatives to thoroughly washing, disinfecting, and drying trailers. Indeed, study 4 demonstrated the value of including washing, disinfecting and TADD in a trailer sanitation protocol. Rather, this work demonstrates the value of possible alternatives when all of the steps cannot be accomplished as a means to reduce the risk of transmitting PEDV between groups of animals during transport.

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