RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of the efficacy of Stalosan F® for inactivating porcine epidemic diarrhea virus (PEDV) in swine feces on the surface of trailers used to haul live pigs – **NPB 13-248**

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INDUSTRY SUMMARY

In May of 2013 porcine epidemic diarrhea virus (PEDV) was detected in swine for the first time in the United States and spread quickly across much of the country, partly due to the movement of contaminated livestock trailers. The objective of this study was to investigate the efficacy of using Stalosan F® disinfectant powder to inactivate PEDV and porcine reproductive and respiratory syndrome virus (PRRSV) in swine feces on metal surfaces similar to what is found in livestock trailers after fecal and other organic matter has been removed by scraping and sweeping, but not washing.

Twenty-four (24), 3-week old barrows, that were negative for PEDV, PRRSV and transmissible gastroenteritis (TGEV), were sourced from a private commercial producer in Iowa. Eight pigs were allocated to one of 3 treatment groups and inoculated via oral gastric tube with 5 mL of either PRRSV and PEDV-negative feces for a negative control (Neg), untreated PRRSV and PEDV-positive feces for a positive control (Pos), or PRRSV and PEDV-positive feces that was treated with Stalosan F (Stalosan). These pigs served as a bioassay to determine the infectivity of virus following treatment. Infectivity was determined by detection of virus with reverse transcriptase polymerase chain reaction (RT-PCR) on fecal swabs collected from the inoculated pigs on days 3 and 7 post-inoculation.

All of the pigs in the Pos (8 of 8) and Stalosan (8 of 8) groups became infected with PEDV. None of the pigs (8 of 8) in the Neg group became infected with PEDV. None of the pigs in the Neg (8 of 8) or Stalosan (8 of 8) groups became infected; however, results for PRRSV were inconclusive since none of the pigs (8 of 8) in the Pos group became infected with PRRSV.

These results suggest Stalosan F did not prevent transmission of PEDV in the presence of feces under conditions representative of a contaminated livestock trailer that has been scraped but not washed.

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KEYWORDS

Swine, PEDV, inactivation, Stalosan F®, disinfectant

SCIENTIFIC ABSTRACT

In May of 2013 porcine epidemic diarrhea virus (PEDV) was detected in swine for the first time in the United States and spread quickly across much of the country, partly due to the movement of contaminated livestock trailers. The objective of this study was to investigate the efficacy of using Stalosan F® disinfectant powder to inactivate PEDV and porcine reproductive and respiratory syndrome virus (PRRSV) in swine feces on metal surfaces similar to what is found in livestock trailers after fecal and other organic matter has been removed by scraping and sweeping, but not washing. Twenty-four (24), 3-week old barrows, that were negative for PEDV, PRRSV and transmissible gastroenteritis (TGEV) negative, were sourced from a private commercial producer in Iowa. Eight pigs were allocated to one of 3 treatment groups and inoculated via oral gastric tube with 5 mL of either PRRSV and PEDV-negative feces for a negative control (Neg), untreated PRRSV and PEDV-positive feces for a positive control (Pos), or PRRSV and PEDV-positive feces that was treated with Stalosan F (Stalosan). These pigs served as a bioassay to determine the infectivity of virus following treatment. Infectivity was determined by detection of virus with reverse transcriptase polymerase chain reaction (RT-PCR) on fecal swabs collected from the inoculated pigs on days 3 and 7 post-inoculation. All of the pigs in the Stalosan (8 of 8) groups became infected with PEDV. This result was not significantly different from the Pos (8 of 8) group ($P>.05$) in which all pigs (8 of 8) also became infected. None of the pigs (8 of 8) in the Neg group became infected with PEDV. None of the pigs in the Neg (8 of 8) or Stalosan (8 of 8) groups became infected, however, results for PRRSV were inconclusive since none of the pigs (8 of 8) in the Pos group became infected with PRRSV. These results suggest Stalosan F did not prevent transmission of PEDV in the presence of feces under conditions representative of a contaminated livestock trailer that has been scraped but not washed.

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 similar outbreaks of diarrhea and significant losses throughout the 1970s and 1980s[4,5]. The PED virus is considered endemic to Europe today, but does not cause widespread significant disease. In parts of Asia similar outbreaks were recognized first in 1982 and have continued to occur since then[4,5]. Until recently, the virus was considered to be absent from the western hemisphere[5,6]. In May of 2013 PEDV was identified for the first time from swine in the United States. The virus caused severe diarrhea in sows and piglets at a commercial sow farm, with near 100% mortality in piglets[6]. Soon afterwards multiple similar cases of severe diarrhea were recognized in sows, suckling piglets, and growing pigs across a wide geographical area of the USA. Outbreaks of PED continue to occur in the USA, with over 6,000 PEDV-positive accessions reported from 29 states as of May 2014[7]. Genetic analysis of PEDV isolates from affected farms in the USA found the virus to be 99%

genetically similar to isolates from China[8-10]. Subsequent genetic analysis of PEDV isolates from multiple other USA farms revealed the presence of two genetically distinct viruses in the USA[11]. Viral cluster analysis suggests both isolates originated in China, but efforts to determine the source of entry to the USA have been unsuccessful.

Although the original mode of entry of PEDV into the USA remains unknown, contaminated livestock trailers certainly represent a significant risk for movement of the virus between and within herds[12]. This is true of other swine diseases as well including porcine reproductive and respiratory syndrome virus (PRRSV)[13].

To mitigate this risk, Stalosan F disinfectant powder has been used by blowing it into soiled hog trailers with an electric leaf blower following a scrape out of the trailer. This represents a significant departure from typical trailer sanitation procedures and should be evaluated. 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)[14].

OBJECTIVES

The objective of this study was to investigate the efficacy of using Stalosan F® disinfectant powder to inactivate PEDV and PRRSV in swine feces on metal surfaces similar to what is found in livestock trailers after fecal and other organic matter has been removed by scraping and sweeping, but not washing.

MATERIALS AND METHOD

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

Source of animals and housing

Twenty-four (24), 3-week old, clinically healthy, barrows were sourced from a private commercial producer in Iowa. At 72 hours after arrival, blood was collected from each pig via jugular venipuncture using a 12 mL syringe with an 18 gauge X 1.5" needle (Monoject™; Covidien, Mansfield, MA) then transferred to an 8.5 mL plastic serum separator tube (BD Vacutainer, 8.5 mL draw; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was centrifuged at 2100 g for ten minutes. Following centrifugation, the serum portion was split into two aliquots and poured off into a five mL snap cap tube (BD Falcon™ polypropylene round-bottom tube; Becton, Dickinson and Company, Franklin Lakes, NJ). One aliquot was frozen and stored at -80° C as a duplicate. Fecal samples were collected by rectal swab using a commercial swab and transport system (Starswabs II; Starplex Scientific Inc., Etobicoke, Ontario Canada). Serum and rectal swabs were submitted to Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) for diagnostic testing. Pigs were negative for PEDV and TGEV (testing rectal swabs) and PRRSV (testing serum samples) using virus-specific reverse-transcriptase polymerase chain reaction (RT-PCR) assays. Animals were PEDV-naïve by serum immunofluorescent antibody testing and TGEV and PRRSV-naïve by serum enzyme-linked immunosorbant assay testing.

On arrival, each pig was identified with a unique plastic livestock ear tag (Allflex USA, Dallas, TX) and weighed. Following a 72 hour rest period and initial screening, as described above, pigs were blocked by weight into three blocks of 8 pigs each. One pig from each block was then randomly assigned to each of three different groups using the RAND function in Excel (Microsoft Corporation, Redmond, WA). Each group was housed in separate rooms, four pigs per room and two rooms per group, in the Iowa State University Veterinary Medical Research Institute for the duration of the study. The four pigs within each room were housed individually in elevated tubs. Each tub was constructed of solid transparent dividers completely separating pigs from one

another. Each divided portion of the tub had dedicated water and feed sources. Pigs were fed ad libitum an un-medicated age-appropriate corn and soybean meal based diet that was free of any porcine origin ingredients. Feces fell through the plastic, slatted flooring of the tub into a common collection area below the pigs, where it flowed by gravity into a holding container that contained water and detergent to contain feces and any PRRSV or PEDV particles and thus reduce the potential for environmental contamination.

Study design

A single group was treated with Stalosan F (Stalosan). In addition, a positive control group (Pos) and negative control group (Neg) were not treated. The Neg group used PRRSV and PEDV-negative feces; all other groups used PRRSV and PEDV-positive feces. See Table 1 for a summary of treatment groups.

The experimental unit was the individual pig. For the bioassay, the inoculum for each pig was prepared using a single aluminum tray that was contaminated with feces and then exposed to the designated treatment.

Challenge material

Challenge material was obtained from two separate studies in which 3-week-old pigs were challenged with PEDV. In one study, forty-eight hours following challenge when pigs were expected to be at peak virus shedding, the pigs were euthanized and feces were collected both ante and post-mortem. In the second study, pigs were euthanized and feces were collected post-mortem 7 days following challenge. Feces from the challenged pigs was pooled and homogenized to ensure uniform challenge material. Feces was placed on ice until it could be frozen at -80°C approximately 1 hour later. Each contributing sample was tested by PEDV RT-PCR at the time of collection to confirm the PEDV-positive status of the feces. The samples were PEDV-positive with cycle threshold (Ct) values of 10.8 and 23.2. After thawing, 5 mL of PEDV-positive feces was mixed with 2mL of porcine reproductive and respiratory syndrome virus (PRRSV) SDSU 73 (3x105 TCID50 per ml).

Feces negative for PEDV was obtained in the same way from the unchallenged pigs in the same two studies and homogenized. Each contributing sample was tested via PEDV RT-PCR at the time of collection to confirm the PEDV-negative status of the feces. Each sample was PEDV-negative with a reported Ct value of >40.

Stalosan F treatment

Prior to treatment, 5 mL of PRRSV and PEDV-positive feces was applied to a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm high sides, and a material thickness of 0.32 cm (6” x 6” x 1” x 1/8”) custom-made to replicate a commercial hog trailer floor. Feces was spread in a thin, even (<=2mm), liquid layer using a disposable, flat adhesive spreader (Lowe's Companies, Inc., Mooresville, NC). This was done for the Stalosan group and the untreated Pos group. The same process was performed for the Neg group, but PEDV-negative feces was used and combined with 2 mL of sterile 0.9% sodium chloride saline (Hospira Inc., Lake Forest, IL) in place of PRRSV. Each treatment replicate used a separate, dedicated tray and spreader to avoid potential cross-contamination between groups. Following application of feces to the aluminum trays, the trays were individually sampled for confirmation of PEDV and PRRSV presence or absence prior to Stalosan F treatment.

Following sampling of the trays, the Pos and Neg trays were sealed with a lid, to prevent accidental treatment with Stalosan F disinfectant powder. Trays were then placed on the bottom deck of a commercial double-decker aluminum hog trailer with dimensions of 49’ 6” x 98” x 51”. The hog trailer was enclosed in plastic to prevent wind from interfering with the dynamics of the Stalosan F powder during the application. Plates were placed evenly throughout the trailer in a randomized block design. The trailer was divided into four equal blocks down its length. Each of these blocks was further divided into two blocks – floor and wall. Trays from each treatment group were evenly distributed between these blocks – one tray per group per block. Tray location within each block was randomized using the RAND function in Excel. Trays that were placed on the wall were hung horizontally so that the plane of the treatment surface is roughly parallel to the plane of the trailer wall at a height of approximately 30” from the floor of the trailer.

Stalosan® F powder was then blown into the trailer using an electric leaf blower (model, Black & Decker, Towson, MD) with an intake tube feeding from a bucket containing Stalosan® F disinfectant powder. Application began at the front of the trailer and progressed to the back of the trailer, moving side to side to evenly distribute the Stalosan F disinfectant powder over all surfaces of the trailer at a rate of 81 grams per square meter. The trailer was placed outside for the application of Stalosan F. The temperature at the time of application was -18 ^{o}C.

Following application of the Stalosan F disinfectant powder, all trays were removed from the trailer and kept at room temperature (20^{o}C) for the duration of the one hour period of contact time.

Bioassay challenge

One hour after the application of Stalosan F, 10 mL saline was applied to each tray in the Stalosan, Pos and Neg groups to suspend the feces for ease of re-collection, and sampled once again to assess the presence or absence of PEDV. The mixture of treated feces and saline was drawn up in a 20 mL syringe, labeled with the id of the specific pig that would be inoculated with the mixture, capped, and set aside. Gloves were worn and changed between each tray during collection to prevent possible cross contamination between trays.

Once all trays within a group had been collected, the material was taken into the respective animal rooms for inoculation of the pigs. Personnel performing the inoculation wore disposable Tyvek coveralls (DuPont, Wilmington, DE) and an N95 respirator (3M, St. Paul, MN) that were changed between groups. Additionally, personnel wore arm-length disposable OB sleeves (Agri-Pro Enterprises, Iowa Falls, IA) and nitrile gloves (VetOne; MWI Veterinary Supply Co., Boise, ID) that were changed between each pig to prevent cross contamination. Following the inoculation of each pig and discarding of the OB sleeves and gloves, the Tyvek coveralls were examined for possible contamination. If any contamination was discovered the coveralls were removed, discarded, and a new pair was donned. Inoculation was performed via gastric gavage with a 14 French rubber catheter (Kendall™; Covidien, Mansfield, MA). Each pig's mouth was held open using a ¾" 45° elbow PVC pipe fitting placed over the restrainer's thumb as a speculum. The catheter was then extended through the esophagus to the pig's stomach for inoculation. Following drenching of the challenge material, ~10 mL of air was injected to clear the catheter of any residual material prior to removal.

Following inoculation, the rectal temperature of the pigs was assessed daily using a digital rectal thermometer that was dedicated to each pig (VetOne®; MWI Veterinary Supply Co., Boise, ID). Clincial signs of diarrhea or other clinical signs were also assessed daily. On days three and seven post-challenge, a rectal swab was collected from each pig and tested for PEDV by RT-PCR. Blood samples were also collected on days three and seven post-challenge and tested for PRRSV by RT-PCR. The use of Tyvek coveralls, masks, gloves, and OB sleeves when sampling pigs was carried out using the same guidelines as when pigs were inoculated with challenge material. During sampling, pigs were not removed from their individual pens to avoid cross contamination between individuals. Swabs and serum from each sampling time point were immediately frozen at -80° C and submitted to the ISU VDL simultaneously for testing by a PEDV N-gene based real-time RT-PCR as previously described[8,12].

Following rectal swabbing and blood collection on day seven post-challenge, all animals were humanely euthanized and necropsied. Gross evaluation was performed of all organ systems. From each pig, fresh cecal and spiral colon contents were collected, fresh and 10% formalin-fixed ileum sections, and fresh and formalin-fixed mesenteric lymph nodes were collected. Fresh samples were immediately frozen at -80° C and all samples were retained in the event further testing might be required to confirm the results obtained by PCR on rectal swabs.

Bioassays were considered to be positive for PEDV if rectal swabs were positive for PEDV by RT-PCR and positive for PRRSV if serum was positive for PRRV by RT-PCR on days 3, 7 or both. For PEDV, a Ct value of ≤ 35 was considered positive. If only one RT-PCR was positive and the other was suspect (Ct > 35 and ≤ 40) or negative, formalin-fixed ileum from these individuals were submitted to the ISU VDL to test for PEDV by immunohistochemical (IHC) staining and microscopic examination. In these instances, IHC results and the presence or absence of histological lesions consistent with PEDV were used to classify the bioassay result as positive or negative.

Statistical analysis (SAS Enterprise Guide 5.1; SAS Institute, Cary, North Carolina, USA) was performed using Fisher's Exact Test to evaluate differences in proportions of positive bioassays between groups with small sample sizes.

RESULTS

Mean RT-PCR values of trays pre- and post-treatment are summarized in Table 2. Because the bioassay results for the Pos group were negative for PRRSV, only results for PEDV are described. All of the trays contaminated with PEDV-negative feces (8 of 8, Neg) were found to be PEDV RT-PCR negative. All trays (16 of 16) that were contaminated with PEDV-positive feces (Pos and Stalosan) were found to be PEDV RT-PCR positive both before and after the treatment period. Ct values from the Stalosan group did increase following contact with Stalosan F for one hour from a pre-treatment mean Ct of 15.2 to 18.4 following treatment. This increase in Ct was not as great in the Pos group (14.4 prior to placement in trailer and 15.4 one hour later).

The results for the bioassay are summarized in Table 3. All pigs (16 of 16) used for the bioassay in both the Pos and Stalosan groups became positive for PEDV by day three, and remained positive through the duration of the trial at day seven. Bioassays were PEDV-negative in all (8 of 8) of the pigs in the Neg group. A 2x2 Fisher's Exact test comparing the proportion of pigs positive by bioassay in the Stalosan and Pos group were significantly different ($P<.001$) compared to the Neg group.

DISCUSSION

A previous study using this bioassay model demonstrated that it is an effective model for evaluation of PEDV inactivation and infectivity.[15] The results suggest that under the conditions evaluated in this study, Stalosan F disinfectant powder did not inactivate PEDV in the presence of feces.

Currently it is estimated that there are not enough livestock trailers or washing facilities in the USA to accommodate the washing of all livestock trailers between every load of swine (T. Burkgren, personal communication, May 08, 2014). Additionally, there is a regional shortage of transporters (J. Hocker, personal communication, May 05, 2014), so it is difficult to shift a transporter's time from hauling swine to washing trailers while still maintaining overall hauling capacity. Washing, disinfecting, and drying times will vary between trailers, facilities, and individual protocols, but a thorough job will require a significant amount of time. A good estimate is that washing and disinfecting will require 2 hours and drying with the use of TADD will require an additional hour, for a total time investment of 3 hours (J. Ellingson, personal communication, April 29, 2014).

At farms, systems, or trucking companies that are unable to wash, disinfect, and dry trailers due to these or other constraints; it may be tempting to implement other means of trailer sanitation such as scraping and applying a disinfectant like Stalosan F. In fact, some haulers were using this strategy in an attempt to reduce the risk of bringing PEDV back to farms on a trailer that had visited a packing plant or other terminal market. The results of this study indicate that this practice may not inactivate PEDV and prevent PEDV infection under the conditions evaluated. When proper washing does occur, it is possible that small amounts of organic material may be left behind on the trailer[16]. The activity of many disinfectants is decreased in the presence of organic

material[17,18]. Additionally, the physical presence of organic material may prevent disinfectant from reaching all surfaces[18]. This becomes especially important in situations where proper washing does not occur, such as the conditions evaluated in this study. The significance of this barrier effect of the feces will depend on the physical properties of the disinfectant being used. A disinfectant product that is liquid and/or has other properties that allow it to penetrate the feces would be affected less by this. Stalosan F, being a dry powder disinfectant would likely be affected by this to a greater degree. This is consistent with post-treatment plate swab PCR results, which indicated that there was a decrease in the amount of virus (increased Ct), but that it wasn't large or complete decrease. This certainly does not prove, but it does support, that there was not adequate contact between the Stalosan F disinfectant powder and the PEDV-contaminated feces.

Further study evaluating other disinfectants with different virucidal and physical properties is needed to help stakeholders fully understand the effect of the presence of fecal material on the efficacy of disinfectants on inactivating PEDV.

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Tables

Table 1. Summary of treatment groups and the conditions they simulate in the field.

Group	Description of Treatment	Treatment Simulates
Neg	PEDV-negative feces and 2 mL of saline were used. Plates were sealed during Stalosan F treatment to prevent contact with disinfectant.	No exposure to PEDV
Pos	PEDV-positive feces and 2 mL of PRRSV isolate were used. Plates were sealed during Stalosan F treatment to prevent contact with disinfectant.	Exposure to a PEDV-contaminated hog trailer with no decontamination attempted.
Stalosan	PEDV-positive feces and 2 mL of PRRSV isolate were used. Plates were left uncovered during Stalosan F treatment to allow contact with disinfectant for one hour.	Exposure to a PEDV-contaminated hog trailer that was manually scraped clean but not washed, with application of Stalosan F disinfectant.

Table 2. Summary of pre- and post-treatment tray swab PEDV RT-PCR assay results

Treatment Group	Pre-Treatment RT-PCR – mean CT (SD)	Post-Treatment RT-PCR – mean CT (SD)
Neg	>40	>40
Pos	14.40 (0.62)	15.35 (0.68)
Stalosan	15.15 (0.39)	18.39 (1.42)

Table 3. Summary of swine bioassay PEDV results by treatment group.

Treatment Group	PEDV RT-PCR CT Values†		Percentage of PEDV positive biosassays (out of 8)
	3 DPI	7 DPI	
Neg	All >40	All >40	0% (0/8) [a]
Pos	15.4	21.5	100% (8/8) [b]
	15.9	21.8	
	17.1	24.0	
	19.4	21.1	
	15.4	26.6	
	16.7	23.9	
	18.0	21.1	
	23.6	24	
Stalosan	15.2	23.2	100% (8/8) [b]
	16.8	22.0	
	17.1	17.1	
	37.8	33.3	
	14.8	19.3	
	32.4	38.0	
	17.3	23.3	
	16.3	20.4	

a, b - Groups with different superscripts indicate statistically significant differences (P <.05) (Fishers Exact Test)

†CT values <35 are considered positive, 35-40 are considered inconclusive, and >40 are considered negative. Bioassays with any inconclusive CT values were confirmed via histopathological exam of ileum sections in conjunction with PEDV IHC.