Evaluation of time and temperature sufficient to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces

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Summary

Objectives: To determine temperature and time applications sufficient to inactivate porcine epidemic diarrhea virus (PEDV) on a commercial livestock trailer, and practical within the constraints of current thermo-assisted drying and decontamination (TADD) capabilities in the industry.

Materials and methods: Thirty-two 4-week-old barrows were inoculated via oral gastric tube with 5 mL of either PEDV-negative feces (Neg; n = 4), untreated PEDV-positive feces (Pos; n = 4), or PEDV-positive feces subjected to 71°C for 10 minutes (71C-10M; n = 4), 63°C for 10 minutes (63C-10M; n = 4), 54°C for 10 minutes (54C-10M; n = 4), 38°C for 12 hours (38C-12H; n = 4), 20°C for 24 hours (20C-24H; n = 4), or 20°C for 7 days (20C-7D; n = 4). These pigs served as a bioassay to determine the infectivity of virus following treatment. Bioassay results were determined by reverse-transcriptase polymerase chain reaction on rectal swabs collected from the inoculated pigs on days 3 and 7 post inoculation.

Results: None of the pigs in the 71C-10M and 20C-7D groups became infected with PEDV. This result differed significantly from that of the Pos group ($P < .05$). Results of the other groups did not differ significantly from that of the Pos group ($P > .05$).

Implication: Holding PEDV in the presence of feces at 71°C for 10 minutes or at 20°C (room temperature) for 7 days is sufficient to inactivate the virus, preventing transmission under the conditions of this study.

Keywords: swine, porcine epidemic diarrhea virus, inactivation, temperature, thermo-assisted drying and decontamination

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Resumen - Evaluación del tiempo y temperatura suficientes para inactivar el virus de la diarrea epidémica porcina en las heces fecales porcinas en superficies metálicas

Objetivos: Determinar la aplicación de temperatura y tiempo suficientes para inactivar el virus de la diarrea epidémica porcina (PEDV por sus siglas en inglés) en un camión de transporte de ganado, y aplicables dentro de las limitaciones de la capacidad actual de descontaminación y secado termo asistido (TADD por sus siglas en inglés) presentes en la industria.

Materiales y métodos: Se inocularon treinta y dos cerdos castrados de 4 semanas de edad vía un tubo gástrico oral con 5 mL de heces negativas al PEDV (Neg; n = 4), heces positivas al PEDV no tratado (Pos; n = 4), o heces positivas al PEDV sometidas a 71°C por 10 minutos (71C-10M; n = 4), 63°C por 10 minutos (63C-10M; n = 4), 54°C por 10 minutos (54C-10M; n = 4), 38°C por 12 horas (38C-12H; n = 4), 20°C por 24 horas (20C-24H; n = 4), o 20°C por 7 días (20C-7D; n = 4). Estos cerdos sirvieron como un bioensayo para determinar la infectividad del virus después del tratamiento. Los resultados del bioensayo se determinaron por medio de la reacción en cadena de polimerasa de transcriptasa reversa en muestras de hisopos rectales recolectadas los días 3 y 7 post inoculación de los cerdos inoculados.

Resultados: Ninguno de los cerdos en los grupos 71C-10M y 20C-7D se infectaron con PEDV. Este resultado difirió significativamente del resultado del grupo Pos ($P < .05$). Los resultados de los otros grupos no difirieron significativamente del resultados del grupo Pos ($P > .05$).

Implicación: Mantener el PEDV en la presencia de heces a 71°C por 10 minutos o a 20°C (a temperatura ambiente) por 7 días es suficiente para inactivar el virus, previniendo la transmisión bajo las condiciones de este estudio.

Résumé - Évaluation du temps et de la température suffisants pour inactiver le virus de la diarrhée épidémique porcine dans des fèces de porc sur des surfaces métalliques

Objectifs: Déterminer les températures et les temps de contact suffisants pour inactiver le virus de la diarrhée épidémique porcine (VDEP) sur une remorque commerciale servant au transport des animaux, tout en étant pratique compte tenu des contraintes

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associées aux capacités de l'industrie porcine en ce qui a trait au séchage thermo-assisté et à la décontamination (TADD).

Matériels et méthodes: Trente-deux mâles castrés âgés de 4 semaines ont été inoculés oralement via un tube gastrique avec 5 mL de fèces négatives pour VDEP (Nég; n = 4), de fèces VDEP positives non-traitées (Pos; n = 4), ou de fèces VDEP positives soumises à une température de 71°C pour 10 minutes (71C-10M; n = 4), 63°C pour 10 minutes (63C-10M; n = 4), 54°C pour 10 minutes (54C-10M), 38°C pour 12 heures (38C-12H; n = 4), 20°C pour 24 heures (20C-24H; n = 4), ou 20°C pour 7 jours (20C-7D; n = 4). Ces porcs ont servi de bio-essai afin de déterminer le pouvoir infectieux du virus suite au traitement. Les résultats du bio-essai ont été déterminés par réaction d'amplification en chaîne par la polymérase à l'aide de la transcriptase réverse sur des écouvillons rectaux prélevés des porcs inoculés aux jours 3 et 7 post-inoculation.

Résultats: Aucun des porcs des groupes 71C-10M et 20C-7D n'est devenu infecté avec le VDEP. Ce résultat différait significativement de ceux du groupe Pos ($P < 0,05$). Les résultats des autres groupes n'étaient pas significativement différents de ceux du groupe Pos ($P > 0,05$).

Implication: Le maintien du VDEP en présence de fèces à 71°C pour 10 minutes ou à 20°C (température ambiante) pendant 7 jours est suffisant pour inactiver le virus, prévenant ainsi la transmission dans les conditions de la présente expérimentation.

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] Porcine epidemic diarrhea virus 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.[4,5] Until recently, the virus was considered to be absent from the western hemisphere.[5,6] In May of 2013, PEDV was identified in swine in the United States for the first time. 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] Outbreaks of PED continue to occur in the United States, with over 6000 PEDV-positive accessions reported from 29 states as of May 2014.[7] Genetic analyses of PEDV isolates from affected farms in the United States found the virus to be 99% genetically similar to isolates from China.[8-10] Subsequent genetic analysis of PEDV isolates revealed the presence of two genetically distinct viruses in the United States.[11] Viral cluster analysis suggests both isolates originated in 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 United States 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] and transmissible gastroenteritis virus (TGEV).[4] Historically, the disease risk posed by contaminated trailers has been effectively mitigated in some cases with the use of trailer washing, disinfection protocols, and thermo-assisted drying and decontamination (TADD) systems.[14] Considering the effectiveness of TADD systems to control these other diseases, and the structural similarity of PEDV to TGEV, TADD may be an efficacious means of inactivating PEDV in contaminated livestock trailers.

The objective of this study was to investigate a range of time and temperature combinations to determine if they are sufficient to inactivate PEDV in swine feces on metal surfaces similar to those found in livestock trailers.

Materials and methods

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

Thirty-two 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, 1.5-inch needle (Monoject; Covidien, Mansfield, Massachusetts) then transferred to an 8.5-mL plastic serum separator tube (BD Vacutainer, 8.5-mL draw; Becton, Dickinson and Company, Franklin Lakes, New Jersey). Blood was centrifuged at 2100*g* for 10 minutes and the serum portion was split into two aliquots and dispensed into two separate 5-mL snap cap tubes (BD Falcon polypropylene round-bottom tube; Becton, Dickinson and Company). One aliquot was frozen and stored at -80°C as a duplicate. Fecal samples were collected using a commercial swab and transport system (Starswabs II; Starplex Scientific Inc, Etobicoke, Ontario, Canada). Serum and fecal samples were submitted to Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) for diagnostic testing. Pigs were negative for PEDV and TGEV (testing fecal samples) and PRRSV (testing serum samples) using virus-specific reverse-transcriptase polymerase chain reaction (RT-PCR) assays. All animals were positive for porcine rotavirus via PCR. Animals were PEDV-naive by serum immunofluorescent antibody testing.

On arrival, each pig was identified with a unique plastic livestock ear tag (Allflex USA, Dallas, Texas) and weighed. Following a 72-hour rest period and initial screening as described above, pigs were blocked by weight into four blocks of eight pigs each. One pig from each block was then randomly assigned to each of eight different groups using the RAND function in Excel (Microsoft Corporation, Redmond, Washington). Each group was housed in a separate room in the Iowa State University Veterinary Medical Research Institute for the duration of the study. The four pigs within each group were housed individually in elevated tubs (Figure 1). Each tub was constructed with solid dividers, completely separating pigs from one another. One of the dividers in each tub was transparent to allow each pig visual contact with one other pig. Each divided portion of the tub had dedicated water and feed sources.

Pigs were fed ad libitum an age-appropriate diet based on corn and soybean meal and free of medications. Feces fell through the plastic slatted flooring of the tub into a common collection area below the pigs, where it fell into a holding container with water and detergent to contain feces and PEDV particles and thus reduce the potential for environmental contamination.

Study design

Combinations of time and temperature evaluated included 71°C for 10 minutes (71C-10M), 63°C for 10 minutes (63C-10M), 54°C for 10 minutes (54C-10M), 38°C for 12 hours (38C-12H), 20°C for 24 hours (20C-24H), and 20°C for 7 days (20C-7D). In addition, a positive control group (Pos) and negative control group (Neg) underwent no time interval or temperature treatment. Prior to exposure to the designated combinations of temperature and time, aluminum trays were covered with feces to simulate a contaminated livestock trailer. The Neg group utilized PEDV-negative feces; all other groups utilized PEDV-positive feces obtained as described. Treatment groups are summarized in Table 1.

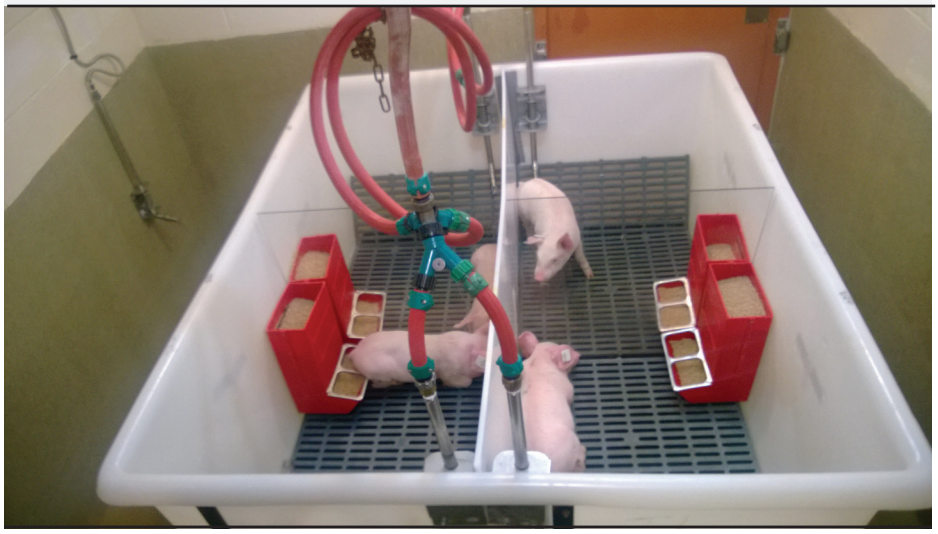
The experimental unit was the individual pig. For the bioassay, the inoculum for each pig was prepared using a single aluminum tray dedicated to that pig. The tray was contaminated with feces and then exposed to the designated combinations of temperature and time.

Challenge material

Challenge material was obtained from a separate study in which 3-week-old pigs were either challenged with PEDV or left unchallenged. 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. Feces from the challenged pigs only were pooled and homogenized to ensure uniform challenge material. After pooling, PEDV-positive feces were split into 5-mL aliquots and stored in 15-mL cone-bottom centrifuge tubes (15-mL Sterile Polypropylene Disposable Centrifuge Tube; Fisher Scientific, Pittsburgh, Pennsylvania) so that each treatment-replicate would have a dedicated sample. The samples were placed on ice until they could be frozen at -80°C approximately 1 hour later. Additional aliquots were obtained for the purpose of assessing the handling and storage process if necessary. One sample was tested via RT-PCR at the ISU VDL prior to freezing to confirm the PEDV-positive status of the feces. This sample was PEDV-positive with a cycle threshold (Ct) value of 10.5.

Feces negative for PEDV were obtained in the same way from the unchallenged pigs and were also split into sufficient aliquots to allow for treatment and testing needs. One aliquot of PEDV-negative feces was tested via PEDV RT-PCR at the time of collection to confirm the PEDV-negative status of the feces. This fecal sample was PEDV-negative with a reported Ct value of > 40.

Figure 1: Elevated tubs used to house pigs for the duration of a study evaluating the ability of different combinations of time and temperature to inactivate porcine epidemic diarrhea virus (PEDV) on metal surfaces similar to those found in livestock trailers. One tub was located in each room. 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. Swine bioassays were used to determine infectivity of virus in the challenge material, and polymerase chain reaction (PCR) of day 3 and day 7 rectal swabs was used to determine bioassay status. The experimental unit was the individual pig, with four pigs per treatment group. Challenge material was prepared using a single aluminum tray, dedicated to one pig, that was contaminated with feces to replicate a contaminated livestock trailer and then exposed to the designated combinations of temperature and time. The Neg group utilized PEDV-negative feces; the other seven groups utilized PEDV-positive feces. Combinations of time and temperature evaluated included 71°C for 10 minutes, 63°C for 10 minutes, 54°C for 10 minutes, 38°C for 12 hours, 20°C for 24 hours, and 20°C for 7 days. In addition, the positive control group (Pos) and negative control group (Neg) underwent no time interval or temperature treatment. Treatment groups are described in Table 1.



Time and temperature treatment

Prior to treatment, 5 mL PEDV-positive feces was applied to an aluminum tray (Figure 2) custom made to replicate a commercial hog trailer floor. Feces were spread in a thin (≤ 2 mm), even, liquid layer using a disposable, flat adhesive spreader. A separate dedicated spreader was used for each tray to avoid cross-contamination between replicates. After application of feces, the trays were individually sampled and tested by PCR to confirm the presence or absence of PEDV RNA prior to the timed temperature treatment.

The treatment was applied to all replicates (n = 4) of a treatment group simultaneously. For treatment groups 71C-10M, 63C-10M, 54C-10M, and 38C-12H, controlled exposure to the designated combination of time and temperature was accomplished using a Fisher Scientific Isotemp Incubator (Fisher Scientific). The incubator was pre-heated to the target temperature for each group prior to placing the trays in the incubator. The surface temperature of the trays was monitored using a Fluke model 53-2-B thermometer with a Fluke model 80PK-1 Type-K bead probe thermocouple (Fluke Corporation, Everett, Washington). Once the average temperature of the four trays had reached the target temperature, timing began.

For treatment groups 20C-24H and 20C-7D, controlled exposure to the designated combinations of time and temperature was accomplished by placing the trays in an insulated cooler that was maintained indoors at room temperature (20°C). The coolers served to insulate the trays from wide variations in temperature that might occur during the diurnal cyclic warming and cooling of the building environment. Temperatures in

Table 1: Time and temperature treatment groups evaluated for their ability to inactivate PEDV on metal surfaces, and the field conditions they simulate *

Treatment name and description	Simulates
Neg	
No treatment, gavage of PEDV-negative feces	Negative control
Pos	
No treatment, gavage of PEDV-positive feces	Positive control
71C-10M	
Heated to 71°C in an incubator, held at 71°C for 10 minutes†	Heating via TADD‡ to a temperature of 71°C, held at 71°C for 10 minutes§
63C-10M	
Heated to 63°C in an incubator, held at 63°C for 10 minutes†	Heating via TADD‡ to a temperature of 63°C, held at 63°C for 10 minutes§
54C-10M	
Heated to 54°C in an incubator, held at 54°C for 10 minutes†	Heating via TADD‡ to a temperature of 54°C, held at 54°C for 10 minutes§
38C-12H	
Heated to 38°C in an incubator, held at 38°C for 12 hours†	Heating to 38°C for 12 hours§¶
20C-24H	
Left at 20°C for 24 hours†	Unused for 24 hours between loads of hogs, but not heated§¶
20C-7D	
Left at 20°C for 7 days†	Unused for 1 week between loads of hogs, but not heated§¶

* Study described in Figure 1.

† PEDV-positive feces (the challenge material) placed on an aluminum tray was subjected to a specified heat treatment and re-collection of feces, which were used to inoculate pigs by gavage to assess PEDV status (bioassay).

‡ Consistent with TADD protocols in some systems[14,15]

§ Simulates pigs exposed to a PEDV-contaminated hog trailer that had undergone decontamination via the specified procedure.

¶ Temperature lower than commonly used in TADD protocols.

PEDV = porcine epidemic diarrhea virus; TADD = thermo-assisted drying and decontamination.

the coolers were monitored with a HOBO temperature data logger (Onset Computer Corporation, Bourne, Massachusetts).

Bioassay challenge

At the expiration of the assigned time, all trays were removed from the incubator or coolers, and 10 mL of sterile 0.9% sodium chloride saline (Hospira Inc, Lake Forest, Illinois) was applied to each tray to suspend the feces for ease of re-collection. Feces were sampled again to assess the presence of PEDV by PCR. The liquid slurry of feces and saline was drawn up in a 20-mL syringe that was capped and labeled with the identification number of the single pig that was to receive the mixture. Gloves were worn and changed between trays during collection to prevent cross-contamination.

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, Delaware) and an N95 respirator (3M, St Paul, Minnesota) that were changed between groups. Additionally, personnel wore arm-length disposable obstetrical sleeves (Agri-Pro Enterprises, Iowa Falls, Iowa) and nitrile gloves (VetOne; MWI Veterinary Supply Co, Boise, Idaho) that were changed between pigs to prevent cross-contamination. After each pig had been inoculated and the obstetrical sleeves and gloves had been discarded, the Tyvek coveralls were examined for contamination. Contaminated coveralls were removed and discarded, and a new pair was donned. Inoculation was performed via gastric gavage with an 18 French rubber catheter (Kendall; Covidien). Each pig's mouth was held open using a ¾-inch, 45° PVC elbow 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. After administration of the challenge material and before removal of the catheter, approximately 10 mL of air was injected to clear the catheter of residual material.

After inoculation, rectal temperatures of the pigs were assessed daily using a digital rectal thermometer dedicated to each pig (VetOne; MWI Veterinary Supply Co). Diarrhea and other clinical signs were also assessed daily. On days 3 and 7 post challenge, a rectal swab was collected from each pig and tested for PEDV by RT-PCR. Tyvek coveralls, masks, gloves, and obstetrical sleeves were used when sampling pigs, employing the same procedures as when pigs were inoculated with challenge material. Pigs were not removed from their individual pens during sampling to avoid cross-contamination between individuals. Swabs from each sampling time point were immediately frozen at -80°C and submitted simultaneously to the ISU VDL to test for PEDV by N-gene-based real-time RT-PCR as previously described.[8,12]

After collection of rectal swabs on day 7 post challenge, all animals were euthanized and necropsied. Gross evaluation of all organ systems was performed and gross pathology noted. From each pig, fresh cecal and spiral colon contents, sections of fresh and 10% formalin-fixed ileum, 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 positive if rectal swabs were PEDV-positive by RT-PCR on days 3 and 7. A Ct value of ≤ 35 was considered positive. If only one RT-PCR result was positive and the other suspect (Ct > 35

Figure 2: Study described in Figure 1. Aluminum trays used to replicate trailer construction materials measured 15.24 ×15.24 cm, with 2.54-cm high sides and a material thickness of 0.32 cm. Feces was applied to the tray (bottom left; 5 mL) and then spread in a thin layer (bottom right).

Table 2: Summary of results of testing pre- and post-treatment tray swabs by RT-PCR assay for PEDV*

Treatment group†	RT-PCR mean Ct (± SD)	
	Pre-treatment	Post treatment
Neg	> 40	NA†
Pos	15.22 (0.73)	NA†
71C-10M	13.40 (0.30)	24.10 (0.76)
63C-10M	13.16 (0.48)	21.56 (0.71)
54C-10M	13.41 (0.32)	20.83 (0.53)
38C-12H	13.28 (1.21)	20.19 (0.09)
20C-24H	14.45 (0.57)	15.07 (0.18)
20C-7D	12.94 (0.55)	17.71 (0.41)

* Study described in Figure 1. Treatment groups and the conditions they simulate described in Table 1. Mean Ct values are summarized for swabs of trays after addition of feces, before and after exposure to temperature or time of exposure.

† As Neg and Pos groups were not exposed to temperature or time treatments, no post-treatment swabs were collected for these groups.

RT-PCR = reverse-transcriptase polymerase chain reaction; PEDV = porcine epidemic diarrhea virus; Ct = cycle threshold; NA = not applicable.

and ≤ 40) or negative, or if the pig died after day 3, formalin-fixed ileum from these individuals was 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) was performed using Fisher's exact test to evaluate differences in proportions of positive bioassays between groups with small sample sizes.

Results

All trays (28 of 28) that were covered with PEDV-positive feces (Pos, 71C-10M, 63C-10M, 54C-10M, 38C-12H, 20C-24H, 20C-7D) were PEDV-positive by RT-PCR before and after exposure to the designated combinations of time and temperature. All trays covered with PEDV-negative feces (four of four; Neg) were PEDV-negative by RT-PCR. Mean RT-PCR values of trays pre-treatment and post treatment are summarized in Table 2.

All replicates that were positive by bioassay across all groups (nine of nine) were positive by day 3, and eight remained positive through day 7. The other pig died prior to day 7.

Bioassays were PEDV-negative in 100% of the pigs (four of four) in the Neg group and in groups 71C-10M and 20C-7D. Bioassays were PEDV-positive in 25% of the pigs (one of four) in groups 63C-10M, 54C-10M, and 20C-10M. Bioassays were PEDV-positive in 50% of the pigs (two of four) in group 38C-12H and in 100% of the pigs (four of four) in the Pos group (Table 3).

A 2 × 8 Fisher's exact test of all groups simultaneously, to evaluate the overall effect of treatment on bioassay outcome, found that treatment did have a significant effect on bioassay status ($P < .05$). More specifically, bioassay outcomes for groups 71C-10M and 20C-7D were significantly different from the Pos group ($P < .05$). No other groups were significantly different from one another (Table 3).

Two animals were removed from the trial early due to illness and death unrelated to infection with PEDV. In both, removal occurred after the day-3 rectal swabs were collected, but prior to day 7. Both pigs were submitted to the ISU VDL for full necropsy and diagnostic workups to determine cause of death and PEDV status. One pig in the

Table 3: Summary of swine bioassay PEDV results by treatment group*

Treatment group†	Mean RT-PCR Ct values‡		PEDV-positive bioassays (%)
	Day 3 post challenge	Day 7 post challenge	
Neg	All > 40	All > 40	0/4[a] (0)
Pos	14.3, 11.4, 10.5, 15.4	18.2, 24.6, 24.4§	4/4[b] (100)
71C-10M	> 40, > 40, > 40, 35.4	All > 40§	0/4[a] (0)
63C-10M	35.7, > 40, 36.2, 13.4	> 40, > 40, > 40, 16.3	1/4[ab] (25)
54C-10M	> 40, > 40, > 40, 18.8	> 40, > 40, > 40, 18.8	1/4[ab] (25)
38C-12H	> 40, > 40, 26.3, 14.1	> 40, > 40, 15.6, 18.1	2/4[ab] (50)
20C-24H	> 40, > 40, > 40, 11.5	> 40, > 40, > 40, 17.1	1/4[ab] (25)
20C-7D	All > 40	All > 40	0/4[a] (0)

* Study described in Figure1. Treatment groups described in Table 1.

† At the time of challenge, n = 4 for all treatment groups.

‡ Ct values ≤ 35 were considered positive; >35 and ≤ 40, suspect; and > 40, negative. Day 3 and 7 swabs were used to determine bioassay status. Bioassays with inconclusive Ct values were confirmed via histopathological examination of ileum sections in conjunction with PEDV immunohistochemistry.

§ One pig in this group died prior to the end of the trial.

[ab] Values within a column with different superscripts are significantly different ($P < .05$; Fisher's exact test).

PEDV = porcine epidemic diarrhea virus; RT-PCR = reverse transcriptase-polymerase chain reaction; Ct = cycle threshold.

positive control group (Pos) was PEDV-positive on day 3 by RT-PCR on feces and was PEDV-positive by RT-PCR on feces and IHC at removal from the study. The other pig in the 71C-10M group was PEDV-negative on day 3 by RT-PCR on feces and PEDV-negative by RT-PCR on feces and IHC at removal from the study. For the pigs not removed early, across all groups, all that were positive by bioassay on day 3 remained positive on day 7 (eight of eight), and all of the pigs that were negative by bioassay on day 3 remained negative on day 7 (22 of 22), Therefore, the bioassay outcomes, as reported in Table 3, for the two pigs removed early were considered to be sufficiently supported and were included in statistical analysis for between-group comparisons.

Discussion

The results of this study suggest that it is possible to inactivate PEDV in the presence of feces by heating trailers to 71°C for 10 minutes or by maintaining surfaces at room temperature (20°C) for at least 7 days. No other combinations of time and temperature evaluated in this study were 100% effective at inactivating PEDV.

The presence of only a single infected pig in three of the treatment groups suggests that the housing system was effective at preventing lateral transmission between pigs. This demonstrates the value of this housing model and associated biosecurity practices for further PEDV swine bioassay research.

Currently it is estimated that there are not enough livestock trailers or washing facilities in the United States to accommodate washing all livestock trailers between loads of swine (Tom Burkgren, DVM, e-mail communication, May 8, 2014). Additionally, there is a regional shortage of transporters (Jason Hocker, DVM, MS, e-mail communication, May 5, 2014), so it is difficult to shift a transporter's time from transporting swine to washing trailers while still maintaining overall hauling capacity. Washing, disinfecting, and drying times will vary among 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 (Josh Ellingson, DVM, MS, oral communication, April 29, 2014).

For farms, systems, or trucking companies that are unable to wash, disinfect, and dry trailers due to the constraints, removing the feces and bedding by scraping and subsequently heating may be practicable. The investigators do not propose that this is a preferred alternative to thoroughly washing, disinfecting, and drying trailers. Rather, this work demonstrates the value of possible alternatives, when washing, disinfection, and drying cannot be accomplished, to reduce the risk of transmitting PEDV between groups of animals. It is important to emphasize that all time measurements in this study began when the samples achieved the target temperature via direct measurement. Variations in contamination level will likely impact the amount of time it takes to achieve the target temperature.

This information may be used to prioritize significant investments in trailer decontamination facilities. If both wash and TADD facilities cannot be built simultaneously, stakeholders will have to decide which is more important. Knowing that heating trailers to 71°C for 10 minutes will inactivate PEDV in the presence of feces may suggest that priority should be given to building TADD facilities.

When washing and disinfection do occur, it is possible that small amounts of organic material may be left behind on the trailer.[15] The activity of many disinfectants is decreased in the presence of organic material.[16,17] Additionally, the physical presence of organic material may prevent disinfectant from reaching all surfaces.[17] In these instances, it is possible that infectious PEDV remains following washing and disinfection. The presence of this potentially infectious material represents a significant biosecurity risk. Inclusion of TADD into trailer decontamination protocols will help to mitigate this risk.

The complexity of trailer design may also prevent disinfectants from reaching all surfaces and all fecal contamination. Livestock trailers are not smooth-side inside, but possess many channels, corners, hinges, and latches, all of which are capable of shielding organic matter from disinfectants. Because heat is transferred directly through metal, TADD would help mitigate this issue. However, this shielding effect of trailer design likely impacts TADD effectiveness to some degree as well. The experimental trays used in this study do not replicate this complexity and so may underestimate the risk of infection in a real-life setting.

This study used experimental group sizes of four pigs per treatment group for economic as well as facility and labor considerations. If a livestock trailer were contaminated with a small amount of infectious organic material, there is potential that many more than four

animals could interact with the material and potentially become infected. For this reason, this study may underestimate the true risk of infection associated with each treatment group.

It is noteworthy that all 24 of the experimental trays that were contaminated with PEDV-positive feces and were exposed to combinations of time and temperature (71C-10M, 63C-10M, 54C-10M, 38C-12H, 20C-24H, 20C-7D) remained positive by RT-PCR following treatment. However, the bioassay results demonstrated that only five of the 24 trays (20.8%) contained an infectious dose of live virus, and 19 (79.2%) did not. This divergence is likely due to differences in virucidal mechanisms that result in viral destruction via membrane disruption, protein denaturation, or deterioration of genetic material.[18] Following exposure to combinations of time and temperature evaluated in this study, a sufficient amount of genetic material remained intact to interact with the primers in a RT-PCR assay. This suggests that viral inactivation occurred via membrane disruption or protein denaturation. In fact, denaturing of viral proteins can occur at higher temperatures such as those described in this study.[18] Additionally, membrane disruption can occur through desiccation of the virus, and it was noted that feces did dry during the heating process. This illustrates that RT-PCR-positive environmental samples of trailers do not necessarily indicate infectious virus is present.

A wide range of temperatures was evaluated in this study to identify effective temperatures at the high end, and ineffective temperatures at the lower end of the range. While this was a good strategy for an initial study, it resulted in a range of temperatures each separated by 7°C or more. Many current TADD facilities operate between 63°C and 71°C.[14] Additionally, at these higher temperatures, significant fuel costs and equipment wear accompany each incremental increase in temperature. Further study evaluating a higher resolution of temperature and time in this range is needed to optimize TADD protocols for inactivating PEDV.

Implications

- Under the conditions of this study, heating scraped, unwashed aluminum trays to 71°C for 10 minutes or allowing them to sit for 7 days at room temperature may be sufficient to prevent transmission of PEDV present in feces as determined by bioassay.
- Under the conditions of this study, exposure to 63°C and 54°C for 10 minutes, 38°C for 12 hours, or room temperature for 24 hours, are not 100% effective at inactivating PEDV in feces.
- Appropriate TADD protocols may be effective at inactivating PEDV in trailers where fecal matter and bedding have been removed by scraping or when some organic matter is present following power washing and disinfection.

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Conflict of interest

None reported.

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