An assessment of sanitation protocols for commercial transport vehicles contaminated with porcine reproductive and respiratory syndrome virus

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Abstract

The objective of this study was to develop and test a rapid (< 2 h) sanitation protocol designed for porcine reproductive and respiratory syndrome virus (PRRSV) positive commercial transport vehicles involving cold water washing and disinfection via fumigation using scale models of weaned pig trailers. The study consisted of 2 phases. Following experimental contamination of model trailers with PRRSV MN 30-100 ($5 \times 10^5 TCID_{50}$), phase 1 evaluated the presence or absence of PRRSV RNA by polymerase chain reaction (PCR) on swabs collected from the trailer interiors 0, 60, and 90 min after treatment. Phase 2 consisted of evaluating the infectivity of trailers 90 min posttreatment by monitoring changes in the PRRSV-status of naïve sentinel pigs housed for 2 h. Treatments included washing only (treatment 1), washing plus formaldehyde fumigation (treatment 2), washing plus fumigation with glutaraldehyde-quaternary ammonium chloride (treatment 3), and washing plus overnight drying (treatment 4). Porcine reproductive and respiratory syndrome virus RNA was detected in all trailers (20 out of 20 replicates) at 60 and 90 min following the application of treatments 1 and 2. These trailers also contained infectious PRRSV, as determined by the infection of naïve pigs housed in treated trailers and the testing of organic debris collected from the interior of trailers by swine bioassay. At 90 min posttreatment, all trailers treated with glutaraldehyde-quaternary ammonium chloride were PCR-negative, non-infectious to sentinel pigs, and swine bioassay negative. Similar results were observed in trailers allowed to dry for 8 h. Under the conditions of this study, it appears certain disinfectants may possess different levels of efficacy against PRRSV and PRRSV-positive models may be effectively sanitized in the absence of overnight drying.

Résumé

Cette étude avait comme objectif de développer et tester un protocole rapide (< 2 h) de sanitation ciblant le virus du syndrome respiratoire et reproducteur porcin (PRRSV) présent sur les véhicules commerciaux de transport utilisant le lavage à l'eau froide et une désinfection au moyen de la fumigation en utilisant des modèles à l'échelle de remorques pour porcs sevrés. L'étude comportait deux phases. Suite à la contamination expérimentale des modèles de remorque avec PRRSV MN 30-100 ($5 \times 10^5 TCID_{50}$), lors de la phase 1 on procéda à évaluer la présence ou l'absence d'ARN du PRRSV par réaction d'amplification en chaîne par la polymérase (PCR) sur des écouvillons prélevés de l'intérieur des remorques 0, 60 et 90 min après les traitements. La phase 2 consistait à évaluer l'infectivité des remorques 90 min post-traitement en suivant les changements dans le statu vis-à-vis le PRRSV chez des porcs sentinelles gardés 2 h dans les remorques. Les traitements examinés étaient : lavage seulement (traitement 1), lavage plus fumigation à l'aide de formaldéhyde (traitement 2), lavage plus fumigation avec glutaraldéhyde-chlorure d'ammonium quaternaire (traitement 3), et lavage plus séchage durant une nuit (traitement 4). L'ARN du PRRSV a été détecté de toutes les remorques (20 fois sur 20 essais) 60 et 90 min suivant l'application des traitements 1 et 2. Ces remorques contenaient également du PRRSV infectieux, tel que déterminé par l'infection de porcs naïfs gardés dans les remorques traitées et l'analyse de débris organiques prélevés à l'intérieur des remorques lors de bio-essais. Au temps 90 min post-traitement, toutes les remorques ayant subit le traitement 3 étaient négatives par PCR, non-infectieuses pour des porcs sentinelles et négatives pour les bio-essais. Des résultats similaires ont été obtenus avec des remorques laissées à sécher pendant 8 h. Sous les conditions expérimentales de cette étude il semble que certains désinfectants possèdent des degrés d'efficacité différents contre le PRRSV et qu'un modèle PRRSV-positif peut être désinfecté efficacement sans période de séchage d'une nuit.

(Traduit par Docteur Serge Messier)

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded enveloped RNA virus classified in the order *Nidovirales*, family *Arteriviridae*, and genus *Arterivirus* (1). Over the years, PRRSV has proven to be a very difficult disease to consistently control over time and across farms. While PRRSV can be eliminated from infected farms through a number of methods, reinfection of farms with a different variant of PRRSV is a frequent event, and the route of viral entry to the farm is often difficult to ascertain.

During the last 2 y, extensive efforts to identify routes of PRRSV transmission between swine farms have been attempted. Reported

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Received February 9, 2004. Accepted April 14, 2004.

routes of PRRSV transmission include infected pigs, semen, contaminated fomites, insects, avian species, and aerosols (2–8); however, a potential route of PRRSV transmission between farms may be the livestock transport vehicle. The process of animal transportation has long been considered to be an important risk factor for pathogen entry into swine farms, through the contact of naïve pigs with contaminated transport vehicles (9). With the advent of multisite production, the economics of finishing pigs in specific regions of North America, and the location of the North American packing industry, transport vehicles carry pigs from farm to farm, across state and international borders, or to the slaughterhouse with increasing frequency. In support of this hypothesis that contaminated transport vehicles are a source of infection, previously published reports have demonstrated how motorized vehicles can act mechanically transport PRRSV over distances of 50 km (10,11).

Recently, a scientific assessment of the role of the transport vehicle in the spread of PRRSV was conducted (12). To enhance study power, multiple scale models (1:150) of weaned pig trailers were constructed that provided an animal density equal to an actual weaned pig trailer capable of transporting 300 pigs (12). Under the conditions of this study, it was demonstrated that PRRSV-naïve swine could become infected with PRRSV through contact with the contaminated interior of the transport models. It was also determined that the concentration of PRRSV required to infect naïve sentinel pigs was $\geq 1 \times 10^3$ $TCID_{50}$, and that allowing the trailer to completely dry after washing effectively prevented infection in 10 out of 10 replicates. However, under field conditions, the accessibility to 80°C water is often limited, and the ability to allow trailers to dry overnight in large swine production systems was difficult to consistently achieve (D. Burns, personal communication December 2003). In large commercial systems, trailer sanitation programs that require time periods greater than 2 h limit the cost-effective use of trailers. Furthermore, in these systems, fumigation to disinfect vehicles that had transported PRRSV-positive swine is increasingly used. However, little scientific information regarding the efficacy of this technique against PRRSV is currently available.

The process of disinfection by fumigation has long been used to disinfect livestock facilities, with the most commonly used fumigant on swine farms being formaldehyde. Formaldehyde has been demonstrated to be effective against enveloped viruses and bacterial spores, with its site of action being cell membranes, enzymes, and nucleic acids. However, its activity is reduced in the presence of organic matter and when dissolved in water (13–15). It also requires prolonged contact time to exert its effect and the fumes can be toxic to personnel (13). Generating gaseous formaldehyde is typically done through the addition of formalin to potassium permanganate, at a rate of 1 L of 37% formalin to 650 g of potassium permanganate per 25 m^3 of building space (15). However, due to issues of personnel and animal safety, several commercial swine operations practice a modified protocol involving the combination of 300 g potassium permanganate with 660 mL of 37% formalin (D. Burns, personal communication September 2003).

Another commercially available disinfectant to fumigate livestock transport vehicles is Synergize (Preserve International, Atlanta, Georgia, USA). Synergize is a combination of 26% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde. Quaternary ammonium compounds are effective against enveloped viruses, and possess limited toxicity; however, their efficacy is reduced in the presence of organic matter (13–15). Glutaraldehydes have long been employed for the process of cold sterilization of surgical instruments, are less affected by organic matter, and require less contact time than formaldehyde (13–15). They act upon cell membranes, enzymes, and nucleic acids, and are effective against enveloped viruses (14).

Table I. Dimensions and parameters of scale model trailers used in the study as compared to full-size trailers

Parameters	Scale model	Full-size trailer
Materials	Aluminum	Aluminum
Structure	Single deck	Single deck
Trailer inventory	2 pigs	300 pigs
Animal density	0.07 sq m/pig	0.07 sq m/pig
Width of trailer	0.28 m	2.14 m
Length of trailer	0.50 m	9.76 m
Height of trailer	0.30 m	2.50 m

Therefore, in order to improve the understanding of the efficacy of fumigation on PRRSV, a sanitation protocol was designed for PRRSV-positive commercial transport vehicles. The protocol incorporated several factors frequently found in commercial swine systems, including cold water for washing, disinfecting via fumigation, and rapid turn-around of trailers (< 2 h). Once developed, it was planned to test the protocol using the scale models.

The study consisted of 2 phases. Phase 1 consisted of swabbing the interior of trailers and testing for PRRSV RNA by polymerase chain reaction (PCR) before and after treatment as an outcome to assess protocol efficacy. Phase 2 consisted of evaluating the infectivity of treated trailers by evaluating changes in the PRRSV-status of naïve sentinel pigs housed for a 2-hour "transport" period.

Materials and methods

Description of model trailers

Throughout the study, the unit of evaluation was the weaned pig trailer. To allow for multiple replications, the University of Minnesota Department of Biosystems and Agricultural Engineering constructed models of weaned pig trailers ($n = 4$). These models were replicates of full-size weaned pig trailers and were built at a scale of 1:150. This scale allowed for an equivalent animal density within the model trailer (2 5-kg pigs at 0.07 m^2/pig) as compared to a full-size weaned pig trailer loaded to capacity (300 5-kg pigs). Similar to the materials found in full-size trailers, the frame, roof, and exterior sidewalls of the model trailers were made of flat metal aluminum, the flooring consisted of polished aluminum tread-plate, and the interior walls were covered with textured styrene and insulated with foil-coated styrofoam. Each of the exterior sidewalls of the models contained openings for proper ventilation and a locking door was available on the end of each model. A comparison of the dimensions of the full-size trailer and the model can be found in Table I.

Source of protocol standards and strain of PRRSV

In an effort to replicate protocols of transport time and sanitation of transport vehicles, data from an international breeding stock

company (Genetiporc, Alexandria, Minnesota, USA) were used throughout the study. This company sells breeding boars and gilts throughout North America and Latin America, operates approximately 15 transport vehicles, delivers approximately 1800 to 2000 truckloads of animals per year and conducts approximately 30 to 35 sanitation procedures per week. As in the other investigations of PRRSV transmission by transport (10–12), the strain of PRRSV employed throughout the study was MN 30-100, a field isolate recovered from a persistently infected sow (16).

Phase 1 of experimental design: Detection of PRRSV RNA

Phase 1 consisted of using the presence or absence of PRRSV RNA, detected by PCR on swabs collected from the trailer interior before and after treatment, to assess protocol efficacy. This phase was conducted on the University of Minnesota Swine Disease Eradication Center (SDEC) research farm. A mechanically ventilated nursery room (25 m^3 in size) was selected for the study, and was heated to 20°C. During the study, the 4 trailer models were placed in adjacent pens in the room (2 trailers per pen). Model trailers were positioned so the rear of the trailer faced the source of the fumigant with the trailer doors fully opened to maximize contact of the fumigant with the trailer interior. During each replicate, the walls, ceilings, and floors of each trailer was contaminated with PRRSV MN 30-100 at a concentration of 5×10^5 $TCID/_{50}$ that had been prepared in 5-mL aliquots of minimum essential medium (MEM) using a hand-operated multi-use power mister (Chapin Manufacturing, Batavia, New York, USA). This high concentration of PRRSV was selected to exceed the previously determined concentration of PRRSV necessary to infect naïve sentinel pigs housed in the model trailers (12) in order to thoroughly test disinfectant efficacy. Prior to contamination, the floors of trailers were covered with wood shavings, a common practice in the North American seed stock industry.

Following contamination, trailers were assigned 1 of 5 treatments. A total of 20 replicates were conducted for each treatment. Twenty replicates allowed for the detection of a 48% reduction in the proportion of infected trailers at a target alpha level of 0.05 and an 80% study power.

Treatment 1 (washing only) — Consisted of a manual scraping of the interior of contaminated trailers to remove soiled bedding (bedding removal), followed by washing the trailer interior with cold water. Scraping of the trailer's interior was done using a hand-held plastic scraper. To insure that mechanical spread of PRRSV did not occur between treated trailers and control trailers, the blade of the scraper was immersed in 70% ethanol, rinsed with sterile water, and swabbed between trailers. Trailers were washed for 72 s using a commercial power-washer (model number TB5030A; American Made Cleaners, Beresford, South Dakota, USA) that provided 21°C water delivered at a pressure of 20 500 kilo Pascals (3000 psi). The 72-second wash time was based on records from the seed stock company reference described earlier indicating that the average time required to wash a full-size weaned pig trailer was approximately 2 h (R. Witt, Genetiporc, personal communication April 2002). Due to the 1:150 differential in size between the full-size trailer and the model trailer, the time used to wash a model trailer was calculated to be 72 s.

Treatment 2 (formaldehyde) — Consisted of bedding removal and washing of contaminated trailers, as previously described, followed by formaldehyde fumigation. The nursery room was heated to 20°C. An aluminum pan containing the 300 g of potassium permanganate was placed approximately 1 m in front of the trailers and 660 mL of 37% formalin was poured carefully into the pan. The room was evacuated, the ventilation system temporarily disabled. A 30-minute period allowing for contact between the fumigant and the trailers was provided.

Treatment 3 (glutaraldehyde-quaternary ammonium chloride) — Consisted of bedding removal and washing of contaminated trailers, as previously described, followed by the delivery of a 1:128 concentration of Synergize using a hurricane fogger (Curtis Dyna-Fog, Westfield, Indiana, USA). The room was heated to 20°C, the fogger was placed approximately 1 m from the trailers, the room ventilation system disabled, and the fogger turned on. As in treatment 2, following the release of all prepared disinfectant, a 30-minute contact period was provided.

Treatment 4 (drying) — Consisted of bedding removal, and washing of contaminated trailers, as previously described in treatments 1 to 3, followed by an 8-hour (overnight) period of drying at 20°C. No disinfecting was conducted.

Controls — Twenty replications of a protocol control were also included in the design. The purpose of the protocol control was to validate that the methods used in treating the trailers did not result in accidental contamination of the models. This consisted of sham-inoculating bedded trailers using MEM that was void of PRRSV. Trailers were then scraped, washed, and fumigated with sterile saline using the fogger, followed by a 30-minute contact period.

Diagnostic monitoring — Following the 30-minute contact period for each treatment, the nursery room was ventilated for 30 min to purge residual disinfectant from the air space. To evaluate the effect of each treatment on trailer sanitation, the interior of each trailer (0.14 cm^2) was swabbed pretreatment (immediately after washing), 60 min posttreatment (30 min contact period plus the 30 min ventilation period), and 30 min later (90 min posttreatment). Prior to sampling, swabs were moistened with MEM, drawn over the walls, floor, and ceiling using a zigzag pattern, placed in sterile plastic tubes (Falcon, Franklin Lakes, New Jersey, USA) containing 2 mL of MEM and frozen at −70°C. Following collection of all required samples, swabs were tested for the presence of PRRSV RNA by PCR. Specifically, the TaqMan PCR assay (Perkin-Elmer Applied Biosystems, Foster City, California, USA) was used (17). After each replicate of each treatment was completed, trailers were rewashed, hand-dried with disposable paper towels, and swabbed to verify that trailers were free of residual PRRSV RNA.

Data analysis — A Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to assess the difference in the number of PCR positive swab samples collected from the treatment groups 1 and 2 and the protocol control.

Phase 2 of experimental design: Infectivity of trailers

Phase 2 consisted of evaluating whether treated trailers were still infective to PRRSV-status of sentinel pigs placed in contact with trailers 90 min following treatment with washing only,

Table II. Summary of diagnostic data from phases 1 and 2

Area tested	Trt 1	Trt 2	Trt 3	Trt 4	Neg Ctrl
Trailer interior pretreatment	20/20[a]	20/20	19/20	20/20	0/20
Trailer interior 60 min posttreatment	20/20	20/20	2/19	NT	0/20
Trailer interior 90 min posttreatment	20/20	20/20	0/19	NT	0/20
Trailer interior allowed to dry (8 h)	NT	NT	NT	0/20	0/20
Number of PRRSV (+) pigs postexposure	2/4[b]	2/4	0/4	0/4	0/4

Trt 1 — Treatment 1, washing only; Trt 2 — Treatment 2, washing plus formaldehyde; Trt 3 — Treatment 3, washing plus glutaraldehyde: quaternary ammonium chloride; Trt 4 — Treatment 4, washing plus overnight drying; Neg Ctrl — Sham-inoculated protocol control; NT — Not tested; PRRSV — Porcine reproductive and respiratory syndrome virus

[a] Number of polymerase chain reaction (PCR)-positive swabs per number of replicates conducted

[b] Number of replicates that demonstrated PRRSV infection of naïve sentinel pigs housed in treated trailers for 2 h per number of replicates conducted

formaldehyde, glutaraldehyde-quaternary ammonium chloride, or drying. This phase was also conducted at the SDEC research farm. Animals were obtained from a source documented as PRRSV-naïve based on 10 y of clinical, diagnostic, and production data. All pigs were 3 wk old and were blood tested to insure a PRRSV-naïve status upon arrival to the study site. Sera were tested for PRRSV-antibodies by the IDEXX ELISA. During this phase, the contamination and treatment protocols of model trailers described in phase 1 were repeated and a total of 4 replicates were conducted. Following completion of each replicate, 1 PRRSV-naïve sentinel pig was housed in a treated trailer for a 2-hour "transport" period. This length of time was based on data from the director of transportation of the reference seedstock company that indicated that the mean period of time required for a shipment of pigs to leave site 1 (breeding, gestation and farrowing farm) and arrive at site 2 (nursery) within the state of Minnesota, USA, was 2 h (R. Witt, Genetiporc, personal communication April 2002). Pigs were placed in the trailers at 90 min posttreatment.

Controls — Four protocol control replicates were also conducted. This involved the use of sham-inoculated (MEM only) trailers that were scraped, washed, and treated with saline using the fogger to insure that accidental contamination of equipment and pigs did not occur.

Swine bioassay — Another means to validate the presence or absence of viable PRRSV in trailer interiors was using a swine bioassay to test the organic debris from trailers of all 4-treatment groups (18). The swine bioassay procedure consisted of administering the sample in question to a naïve pig via intramuscular injection followed by assessment of whether a change in the PRRSV status of the naïve sentinels occurred. To prepare the sample, organic debris (residual wood shavings) that remained in the interior of trailers in groups 1 to 4 was collected immediately posttreatment using a pair of forceps. Debris from trailers was pooled according to treatment (4 pools, 1 pool per treatment group) into 1 of 4 plastic Falcon tubes each containing 10 mL of MEM, and centrifuged at 4200 $\times$ *g* for 10 min. Each of the 4 treatment pools was divided in half and a 5 mL aliquot of the supernatant was injected into an individual pig (2 pigs per treatment pool). Pigs were then monitored for changes in their PRRSV status. Two sham-inoculated (MEM only) negative controls were conducted as well. Forceps were dipped in 70% ethanol, flamed, and cooled in sterile saline between treatment groups.

Diagnostic monitoring — Following the 2-hour period, each pig was removed from a trailer and tested for PRRSV RNA by PCR on days 3 and 7 postexposure. Pigs were placed in individual pens, allocated to separate rooms according to treatment and control groups. Bioassay pigs were housed and tested in a similar manner. Nose-to-nose contact between pigs was prevented at all times. Biosecurity measures were put in place to prevent the spread of PRRSV between rooms (4,19). These protocols included changing disposable boots, gloves, and coveralls between rooms, and 5-second immersion of boots in 6.5% sodium hypochlorite boot baths upon entering each room. Between replicates, trailers were washed, disinfected, and dried as in phase 1, and swabbed to document the absence of residual PRRSV between replicates.

Results

Phase 1

The results from the trailer interior swabs collected pretreatment (immediately postwashing) and at 60 and 90 min posttreatment are summarized in Table II. A total of 80 swabs were collected prior to treatment across the 20 replicates of each of the 4 treatments. Porcine reproductive and respiratory syndrome virus-RNA was detected on 79 out of 80 swabs, with the single negative postwash swab collected during replicate number 3 from the treatment 3 groups. Porcine reproductive and respiratory syndrome virus-RNA was detected in 20 out of 20 swabs collected at both 60 and 90 min following treatments 1 (washing only) and 2 (formaldehyde). In contrast, only 2 out of 19 swabs were PCR-positive at 60 min following treatment 3 (glutaraldehyde-quaternary ammonium chloride) and 0 out of 19 swabs at 90 min posttreatment. All 20 swabs collected from trailers allowed to dry for 8 h (treatment 4) and protocol controls were negative. All swabs collected from trailers that had been rewashed and hand dried with disposable paper towels between replicates were PCR negative.

Data analysis — Kruskal-Wallis non-parametric ANOVA analysis of the difference in the number of PCR positive swab samples

collected from treatment groups 1 and 2 compared to the number of positive swabs collected from treatment groups 3, 4, and the protocol control was statistically different ($P < 0.001$).

Phase 2

Two of 4 pigs became infected with PRRSV following exposure for 2 h to contaminated trailers that were washed only (treatment 1) on day 3 and 7 postinoculation (Table II). Two of 4 pigs were also PCR-positive on both testing days following exposure to trailers treated with formaldehyde (treatment 2). In contrast, all 4 pigs remained PRRSV-naïve following contact with trailers treated with glutaraldehyde-quaternary ammonium chloride, or those allowed to dry overnight (treatments 3 and 4, respectively). All protocol control replicates were negative.

In regards to the swine bioassay, 2 out of 2 pigs injected with supernatants from wood shavings collected from trailers treated with washing only or formaldehyde were PCR-positive on days 3 and 7 postinoculation. All pigs injected with supernatant from trailers treated with glutaraldehyde-quaternary ammonium chloride, or those allowed to dry overnight were PCR-negative on both days, while negative control replicates remained negative. Adverse side effects (irritation of the skin, abscess formation, swelling) were not detected at the site of injection of the bioassay sample, nor did pigs experience fever or loss of appetite.

Discussion

The objective of this study was to develop and test a PRRSV sanitation protocol for commercial livestock vehicles. Key components of this protocol were the use of cold water for washing, disinfection via fumigation, the absence of drying, and completion of the protocol in < 2 h. The study utilized scale models of weaned pig trailers to enhance replication of selected treatments. Outcomes measured included the presence or absence of PRRSV RNA, as detected on swabs collected from the trailer interior posttreatment, and the evaluation of PRRS status of naïve sentinels that were housed in treated trailers.

Results suggest that under the conditions of the study, the use of cold water for washing had little impact on eliminating PRRSV from in trailer interiors. Porcine reproductive and respiratory syndrome virus RNA was detected on 99% (79 out of 80) of the swabs collected immediately after the washing procedure across all replicates. Also, it did not appear to be beneficial for the complete removal of organic debris from the trailer interior, since after the 72-second washing, small amounts of residual bedding were visible in all of the trailers, a frequent observation under field conditions.

Also, through the use of the model, certain treatments appeared to demonstrate different degrees of efficacy against PRRSV. Porcine reproductive and respiratory syndrome virus RNA was not detected by PCR in any of the replicates where trailer models were treated with glutaraldehyde-quaternary ammonium chloride or those allowed to dry overnight. The difference in the number of positive swabs collected from trailers in these groups versus the number collected from trailers in treatment groups 1 and 2 was significant ($P < 0.001$). One explanation for these results may be the combination of the 2 disinfectants, both known to be efficacious against enveloped viruses, while glutaraldehyde requires less contact time than formaldehyde and is less affected by organic matter (13). The efficacy of overnight drying has been documented for eliminating PRRSV from contaminated trailers and the results from treatment group 4 support the previously published data (12).

In contrast, the efficacy of formaldehyde fumigation appeared to be very poor, with PRRSV RNA being detected in 20 out of 20 replicates at 90 min posttreatment, similar to the levels found in trailers in group 1 that were only scraped and washed. However, it must be emphasized that the protocol used in this study differed from previously published protocols used to fumigate swine facilities (15). This protocol used smaller quantities of potassium permanganate and formalin, and this may have resulted in a reduced concentration of formaldehyde gas available to disinfect trailers. It is well documented that a linear relationship exists between the concentration of formaldehyde and pathogen killing rate (13) and this could have been negatively impacted by the reduced amounts of chemical compounds. This protocol also provided a very short period of contact between the gas and the trailer (90 min), whereas protocols for the fumigation of facilities require a minimum contact period of 10 to 12 h (15). Finally, the efficacy of formaldehyde is known to be reduced when dissolved in water (15), and following washing, excessive pooling of water was frequently observed on the tread plate floor of the models, similar to what can be seen in an actual trailer.

As with all scientific studies, this study contained several acknowledged limitations. The primary limitation of the phase 1 design was the inability to determine if viable PRRSV was actually present in the trailer interior posttreatment. Without the use of confirmatory tests, such as virus isolation or swine bioassay, it was impossible to conclusively determine whether a positive PCR result was indicative of live or dead virus. Furthermore, a negative PCR result could be due to multiple factors, including the diagnostic sensitivity of the test, degradation of viral RNA in the sample through prolonged contact with the disinfectant, interference of the disinfectant with the PCR assay, or the results of a truly efficacious disinfectant that not only rendered the virus inactive, but also degraded its nucleic acid. To better evaluate the true meaning of the PCR result, the phase 2 trials were conducted.

Regarding the interpretation of a positive PCR result, recovery of PRRSV from sentinel pigs exposed to trailers treated with formaldehyde indicates the presence of infectious, viable PRRSV posttreatment, a conclusion that is further supported by the results of the swine bioassay. In contrast, infectious PRRSV was not detectable in trailers treated with glutaraldehyde-quaternary ammonium chloride in any of the methods employed. Regarding the interpretation of a negative PCR result, based on the ability of the TaqMan PCR assay to regularly detect PRRSV RNA in numerous samples, test sensitivity did not appear to be an issue. Furthermore, this assay has a reported level of detection of 0.01 $TCID_{50}$ per PCR reaction (17). In regards to the possibility of degradation of PRRSV RNA in swab samples secondary to prolonged contact with disinfectant during storage, this did not appear to be a problem, based on the large number of PCR positive samples detected at 60 and 90 min posttreatment across treatments 1 and 2. Since it was not possible in this study to add a compound to the sample to neutralize the disinfectant due to the potential virucidal effects of exogenous chemicals (14),

samples were stored immediately postcollection at −20°C to retard disinfectant activity (13), and then underwent RNA extraction within 24 h postcollection. As for the potential interference of the individual disinfectants with the PCR assay, this did not appear to be an issue. Porcine reproductive and respiratory syndrome virus RNA was successfully detected in 20 out of 20 and 2 out of 19 replicates at 60 min following application of treatments 2 and 3, respectively, indicating that it was possible for the TaqMan PCR assay to properly function in the presence of residual disinfectant. Therefore, the combined results from the phase 1 and 2 trials suggest that under the conditions of this study, the glutaraldehyde-quaternary ammonium chloride treatment was highly efficacious against PRRSV if allowed to contact contaminated trailer surfaces for a minimum of 90 min. However, it must be remembered that this study was conducted at a temperature of 20°C, and since disinfectant activity is reduced with decreasing temperature (13–15), it is not known if similar results would be observed at cooler conditions.

Despite these interesting findings, it must be remembered that this study has other acknowledged limitations, the most obvious being the inability to use full-size trailers and large loads of pigs. The models are based on weaned pig trailers and their construction design does not mimic a trailer that transports market animals, variables that certainly could impact the level of contamination in the trailer interior and the ease of cleaning. Therefore, these protocols need further evaluation in full-size trailers, both of the weaned pig and market swine designs. A high concentration of PRRSV was also used to contaminate the trailers and it is not known if this level of contamination is representative of actual transport conditions. The entire interior of the models were contaminated, and this may not be representative of field conditions. Also, the size of the swab was not proportional to the size of the model trailer and this may have impacted the recovery of PRRSV RNA. However, it has been previously determined that sentinel pigs can be infected with PRRSV in the model trailers when models are contaminated with concentrations of $\geq 1 \times 10^3$ $TCID_{50}$. Therefore, in order to test the efficacy of the decontamination protocol, a high concentration was desired. The study was also conducted using a specific age of pig; it was not possible to conduct the study using market age animals or adult breeding swine. Also, despite the fact that a relatively large number of replicates were conducted in phase 1, this is not a sufficient number of replicates to predict the frequency of the events recorded in the study. Furthermore, since only 4 replicates involved the use of live animals, because of this small sample size, no estimation can be made regarding the frequency of the reported events. Finally, it was not possible to quantify the amount of PRRSV RNA present in PCR positive samples; however, in phase 2, there did appear to be sufficient quantity of virus to infect some of the sentinel pigs. Therefore, a future objective may be to sample and quantify the actual concentration of PRRSV in commercial trailers since quantitative PCR assays are becoming available in certain diagnostic laboratories. It must also be emphasized that the results of this study cannot be extrapolated to other swine pathogens, such as transmissible gastroenteritis virus or *Mycoplasma hyopnuemoniae*, and further testing is required using alternative agents before such claims can be made.

Despite these limitations, the study had much recognizable strength. This study re-enforced the value of drying commercial livestock vehicles for inactivation of PRRSV (12). The information brought about a new understanding in regards to the efficacy of potential PRRSV-sanitation protocols for commercial transport vehicles, information that was lacking in the industry prior to this investigation. As before (12), the use of scale models allowed for frequent replication of each treatment, an essential component of all transmission studies. While it was true that the trailer size and pig numbers were small, the models provided equivalent animal densities to that of a full-size trailer. Furthermore, it would have been impossible to obtain full-size trailer loads (200 to 300 pigs) for even a single replicate, much less to repeat the study at any frequency. Finally, whenever possible, industry standards for transport times and wash water temperatures, pressures, as well as disinfecting products and practices were used to replicate real-world situations, including the presence of organic debris after washing. In many instances in actual transport vehicles, residual wood shavings can be observed after washing. While the use of detergents may reduce the frequency of this event, these products were not included in this study.

In conclusion, this study supports the claim of A. Mateos Poumian, regarding PRRSV, that "All trucks, trailers, and other vehicles used for transporting animals, animal products, products, feed, offal, and contaminated equipment are a potential risk in the spread of disease" (9). Therefore, based on the information generated through these experiments, is hoped that swine producers and practitioners will continue to understand and appreciate the merit of sanitizing livestock transport vehicles. However, it is also suggested that while these results demonstrate efficacy against PRRSV in the absence of drying, whenever possible, a complete and through drying of the livestock transport vehicles is still the best means to safeguard recipient farms

Acknowledgments

Funds for this study were made available by Genetiporc LLC, Alexandria, Minnesota, USA, and the Utah Pork Producers Association.

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