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Evaluating a Dry vs. Wet Disinfection in Boot Baths on Detection of Porcine Epidemic Diarrhea Virus and Porcine Reproductive and Respiratory Syndrome Virus RNA

Olivia L. Harrison, Grace E. Houston,[1] Allison K. Blomme,[1] Haley K. Otott,[1] Jianfa Bai,[1] Elizabeth G. Poulsen Porter,[1] Jason C. Woodworth, Chad B. Paulk,[1] Jordan T. Gebhardt,[2] Cassandra K. Jones

Summary

Maintaining biosecurity between swine barns is challenging, and boot baths are an easily implementable option some utilize to limit pathogen spread. However, there are concerns regarding their efficacy, especially when comparing wet or dry disinfectants. The objective of this study was to evaluate the efficacy of boot baths in reducing the quantity of detectable porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) genetic material using wet or dry disinfectants. Treatments included 1) control; 2) dry chlorine powder (Traffic C.O.P., PSP, LLC, Rainsville, AL); and 3) wet quaternary ammonium/glutaraldehyde liquid (1:256 Synergize, Neogen, Lexington, KY). Prior to disinfection, rubber boots were inoculated with 1 mL of co-inoculants of PRRSV (1×10^5 $TCID_{50}$/mL) and PEDV (1×10^5 $TCID_{50}$/mL) and dried for 15 min. After the drying period, a researcher placed the boot on the right foot and stepped directly on a stainless steel coupon (control). Alternatively, the researcher stepped first into a boot bath containing either the wet or dry sanitizer, stood for 3 s, and then stepped onto a steel coupon. After one min, an environmental swab was then collected and processed from each boot and steel coupon. The procedure was replicated 12 times per disinfectant treatment. Samples were analyzed using a duplex qPCR at the Kansas State Veterinary Diagnostic Laboratory. Cycle threshold values, which indicate the presence or absence of the inoculants and their relative concentrations when present, were analyzed using SAS GLIMMIX (v. 9.4, SAS Institute, Inc., Cary, NC). There was no evidence of a disinfectant × surface × virus interaction ($P > 0.10$). An interaction between disinfectant × surface impacted ($P < 0.05$) the quantity of detectable viral RNA. As expected, the quantity of the viruses on the coupon were greatest in the control, indicating that a contaminated boot has the ability to transfer viruses from a contaminated surface to a clean surface. Compar-

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atively, the dry disinfectant treatment resulted in no detectable viral RNA on either the boot or subsequent coupon. The wet disinfectant treatment had statistically similar ($P > 0.05$) viral contamination to the control on the boot, but less viral contamination compared to the control on the metal coupon. In this experiment, a boot bath with dry powder was the most efficacious in reducing the detectable viral RNA on both boots and subsequent surfaces.

Introduction

Disease spread between populations of animals is a major concern for many swine producers. Protocols like changing clothes and wearing plastic boot covers help reduce farm-to-farm disease spread between production sites.[3,4] However, reduction of room-to-room disease spread within a single production site is typically limited due to challenges in infrastructure and practicality of implementation. One easily implemented option is to place a boot bath between rooms with the intent to sanitize the boot bottoms of personnel as they move from one room to another.

The efficacy of the boot bath in a production system is dependent on the disinfectant utilized, the pathogen of concern, and the maintenance of the system itself. Boot baths have been demonstrated to be effective at preventing fomite transmission of porcine reproductive and respiratory disease syndrome (PRRSV).[4] However, their usefulness is often questioned due to the maintenance needed to maintain efficacy.[5] Historically, most boot baths have contained wet sanitizer, which can pose a slip hazard and quickly accumulates organic matter, potentially reducing its efficacy over time. Alternate dry powder disinfectants have recently become available, but there are little data to compare the efficacy of the dry powder compared to the wet disinfectant. Therefore, the objective of this study was to evaluate the efficacy of boot baths, using either wet or dry disinfectants, on the detectability of porcine epidemic diarrhea virus (PEDV) and PRRSV genetic material.

Procedures

All experimental procedures were approved by the Institutional Biosafety Committee at Kansas State University (IBC #1511) and were conducted in the Cargill Feed Safety Research Center (FSRC) at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Preparation of inoculum

Prior to the experiment, 4 mL of 1.33×10^6 $TCID_{50}$/mL PEDV (USA/Co/2013) and 4 mL of 1.33×10^6 $TCID_{50}$/mL PRRSV (1-7-4) were individually diluted with 36 mL phosphate buffer solution (PBS) in separate containers for an approximate final concentration of 1×10^5 $TCID_{50}$/mL. Viruses were further divided into 10 mL aliquots and stored at -112°F until the start of the experiment.

[3] Otake, S., S. A. Dee, K. D. Rossow, J. Deen, H. S. Joo, T. W. Molitor, and C. Pijoan. 2002. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). J. Swine Health Prod. 10:59-65.

[4] Dee, S., J. Deen, and C. Pijoan. 2004. Evaluation of 4 intervention strategies to prevent the mechanical transmission of porcine reproductive and respiratory syndrome virus. Can. J. Vet. Res. 68:19-26.

[5] Bashandy, E. Y., S. A. Nasef, S. A. E. Nasr, M. F. AbdEIAty, and O. M. K. Zahran. 2017. Efficacy of a novel foot pan in biosecurity protocols for control of salmonellae in poultry farms. J. Vet. Med. Res. 24:28-40. doi: 10.21608/jvmr.2017.43260.

Preparation of surfaces

At the start of the experiment, aliquots of each virus were thawed in a biosafety cabinet within the BSL-2 facility. Next, 9 mL of each virus was combined in a single container and gently agitated to create a single container with 18 mL of a PEDV/PRRSV co-inocula. From this container, 1-mL aliquots were drawn into individual syringes and stored in the biosafety cabinet.

Meanwhile, boots, boot baths, and surfaces were prepared within the BSL-2 facility but outside the biosafety cabinet. Thirty-six boots (size 12, right foot only) were placed upside down on a boot drying rack and dusted with autoclaved ground corn to disrupt the rubber surface tension of the boot prior to viral inoculation. Twenty-four plastic containers (14 in. × 10 in. × 4 in.) were filled with approximately 1 in. of either dry disinfectant (Traffic C.O.P., PSP, LLC, Rainsville, AL) or wet disinfectant (Synergize, Neogen, Lexington, KY). The dry disinfectant was a dry powder containing chlorine, silicates, and acid-impregnated zeolites used directly as received from the manufacturer. The wet disinfectant was a quaternary ammonium/glutaraldehyde liquid that required 1:256 dilution with water per the manufacturer's directions. Thirty-six stainless steel coupons (4 × 4 in.) were autoclaved and placed at least 4 in. apart from one another.

Surface inoculation

One mL of the co-inoculant was distributed in the same location across the sole of each boot. Boots were then allowed to air-dry for 15 min at ambient temperature and humidity. After the drying period, a single designated researcher placed the boot on the right foot and stepped directly on a stainless-steel coupon (control). Alternatively, the researcher stepped first into a dry or wet boot bath and stood for 3 s before stepping onto the steel coupon. Boots were then placed back on the drying rack and surfaces were allowed to air-dry for 1 min at ambient temperature and humidity. Next, an environmental swab was collected and processed from each boot and steel coupon using procedures described by Elijah et al. (2021b). These procedures were repeated 12 times. Altogether, 72 environmental swabs were collected, representing 12 replicates of 3 boot bath treatments (control, dry disinfectant, or wet disinfectant) and two surfaces (rubber boot and stainless-steel coupon).

Quantitative viral analysis

Environmental swabs were analyzed for quantitative real-time polymerase chain reaction (qRT-PCR) for PEDV and PRRSV at the Kansas State University Veterinary Diagnostic Laboratory using procedures similar to those described by Elijah et al. (2021a).[6] First, 50 µL of supernatant was placed in a deep well plate and RNA extracted using a Kingfisher Flex magnetic particle processor (Fisher Scientific, Pittsburgh, PA) and a MagMAX-96 Viral Isolation Kit (Life Technologies, Grand Island, NY). The final elution volume was reduced to 60 µL, and extracted RNA was stored at -112°F until analyzed for PEDV or PRRSV using a qRT-PCR duplex assay with a maximum cycle threshold of 45. Results were reported as the number of samples considered positive and the cycle threshold (Ct) at which either PEDV or PRRSV RNA was detected.

[6] Elijah, C.G., C. K. Jones, C. Evans, H. K. Wecker, C. R. Stark, J. Bai, E. G. Poulsen-Porter, A. K. Blomme, J. C. Woodworth, C. B. Paulk, J. T. Gebhardt. 2022. Quantification of decontamination strategies for semi-truck cabs. Proceedings of the 53rd AASV Annual Meeting. 37-40. doi: 10.54846/am2022/5.

Statistical analysis

Results were analyzed as a split plot design with boot bath pan as the main experimental unit and surface (either boot or coupon) as the sub-plot using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Fixed effects included disinfectant (control, dry, or wet), surface type (boot or steel), virus (PEDV or PRRSV), and their associated interactions. Random effect included boot bath pan. Two response criteria were considered, the proportion of PCR positive samples and the quantity of detectable viral RNA. To estimate the proportion of PCR positive samples, the number of samples with detectable PEDV or PRRSV RNA was placed in ratio to the number of total samples. Data were analyzed by fitting to a binary distribution, logit link, Laplace approximation, and ridge-stabilized Newton-Raphson algorithm. As a binary distribution model, data were fit by each individual interaction, starting with the disinfectant × surface type × virus interaction, and their subsequent main effects. To estimate the quantity of detectable viral RNA, the Ct of each sample was used. If no PEDV or PRRSV RNA were detected, samples were assigned a value of 45.0. A Kenward-Roger denominator degree of freedom adjustment was used, as well as a Tukey-Kramer multiple comparison adjustment. Results were considered significant at $P \leq 0.05$.

Results and Discussion

There was no evidence of a disinfectant × surface × virus interaction ($P > 0.05$) for either the proportion of positive samples or their quantity of detectable viral RNA (Table 1). However, there was a disinfectant × surface interaction ($P < 0.05$) for both response criteria. There was no evidence ($P > 0.05$) that the proportion of PCR positive samples differed between samples collected from boots or steel coupons for the control treatment or the boots for the wet disinfectant treatment. However, these all had a greater ($P < 0.05$) proportion of PCR positive samples than the steel surface after the boot bath with wet disinfectant. There were no PCR positive samples for either the boot or steel surface after the boot bath with dry disinfectant. The quantity of viral RNA was greater ($P < 0.05$) for the boots and steel coupons from the control treatment, and the boots from the wet disinfectant treatment, as compared to the steel coupons from the wet disinfectant treatment and either surface from the dry disinfectant treatment (Table 1).

In addition to the disinfectant × surface interaction reported, the quantity of detected viral RNA in this study was also affected by a disinfectant × virus interaction ($P < 0.05$; Table 2). Specifically, there were greater ($P < 0.05$) quantities of PEDV detected in the control samples than of PRRSV in the control or PEDV in samples from the boot bath with wet disinfectant. Again, no PEDV or PRSSV was detected in samples from the boot bath with dry disinfectant.

Boot baths are an easily implemented biosecurity measure to reduce room-to-room viral transfer on swine farms and other facilities. A boot bath containing a dry chlorine powder in this experiment surpassed the performance of a boot bath containing a wet quaternary ammonium/glutaraldehyde liquid disinfectant. While the wet disinfectant reduced the quantity of viral RNA compared to the control, it did not reduce viral RNA of either virus beyond detectable limits. However, one of the major challenges of using boot baths is the buildup of organic matter during use, which may impact sanitizer efficacy. Furthermore, these results only report the quantify of detected viral RNA, not the infectivity of these samples in live animals in production settings. Future

research should focus on the utilization of dry disinfectant in production settings and in the presence of organic matter, as well as to evaluate viral infectivity.

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Table 1. Detection of viral RNA on boots or subsequent steel surfaces after stepping in a boot bath containing a wet or dry disinfectant[1]

	Boot bath disinfectant type		
Item	**Control**	**Dry**	**Wet**
PCR positive[2]			
Boot	19/24[c]	0/24[a]	21/24[c]
Steel	22/24[c]	0/24[a]	9/24[b]
Ct[3]			
Boot	37.0[c]	45.0[a]	38.1[c]
Steel	34.0[d]	45.0[a]	42.2[b]

[1]Boots were inoculated with 1 mL of a PEDV/PRRSV co-inoculant and were randomly subjected to one of three boot bath disinfectants. Boots were stepped onto a stainless-steel coupon (4 × 4 in.) after submersion in the boot bath. The dry disinfectant was a powder containing chlorine, silicates, and acid-impregnated zeolites (Traffic C.O.P., PSP LLC, Rainsville, AL). The wet disinfectant was liquid quaternary ammonia and glutaraldehyde blend (1:256 dilution; Synergize, Neogen, Lexington, KY). Samples with no detectable RNA were assigned a Ct value of 45.0. Disinfectant × surface × virus, $P > 0.05$.

[2]PCR positive: Disinfectant × surface, $P = 0.015$.

[3]Ct is the average cycle threshold value for both PEDV and PRRSV. Disinfectant × surface, $P = 0.0001$; SEM = 0.61.

[abcd] Means with differing superscripts differ significantly.

Table 2. Detection of PEDV and PRRSV RNA after stepping in a boot bath containing a wet or dry disinfectant[1]

	Boot bath disinfectant type		
Item	**Control**	**Dry**	**Wet**
PCR positive[2]			
PEDV	20/24	0/24	19/24
PRRSV	21/24	0/24	11/24
Ct[3]			
PEDV	34.0[d]	45.0[a]	38.0[c]
PRRSV	37.0[c]	45.0[a]	42.3[b]

[1]Boots were inoculated with 1 mL of a PEDV/PRRSV co-inoculant and were randomly subjected to one of three boot bath disinfectants. Boots were stepped onto a stainless-steel coupon (4 × 4 in.) after submersion in the boot bath. The dry disinfectant was a powder containing chlorine, silicates, and acid-impregnated zeolites (Traffic C.O.P., PSP LLC, Rainsville, AL). The wet disinfectant was liquid quaternary ammonia and glutaraldehyde blend (Synergize, Neogen, Lexington, KY). Samples with no detectable RNA were assigned a Ct value of 45.0. Disinfectant × surface × virus, $P > 0.05$.

[2]PCR positive: Disinfectant × virus, $P > 0.05$.

[3]Ct is the average cycle threshold value for both boot and stainless-steel surfaces. Disinfectant × virus, $P = 0.0019$; SEM = 0.66.

[abcd] Means with differing superscripts differ significantly.