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Evaluation of the Efficacy of Disinfectant Footmats for the Reduction of Bacterial Contamination on Footwear in a Large Animal Veterinary Hospital

K.J. Hornig, B.A. Burgess, N.T. Saklou, V. Johnson, A. Malmlov, D.C. Van Metre, P.S. Morley, and S.R. Byers

Background: Infection control is critical to providing high-quality patient care. Many veterinary teaching hospitals (VTHs) utilize footbaths or footmats at entrances and key control points throughout the facility to decrease trafficking of pathogenic microorganism on contaminated footwear.

Hypothesis/Objectives: To compare efficacy of 4 disinfectants used in footmats for decreasing bacterial contamination of footwear in a large animal hospital.

Animals: A single adult dairy cow was housed in a stall for 4 days to facilitate stall contamination with fecal material.

Methods: Overboots were experimentally contaminated with organic material in a standardized manner. Each boot was randomly assigned to 1 of 5 treatments (no treatment, or exposure to 1 of 4 disinfectants: an accelerated peroxygen [AHP], a peroxygen [VIRKON], a quaternary ammonium [QUAT], and a phenolic disinfectant [PHENOLIC]) by stepping on a soaked footmat and collecting samples from boot soles. Generalized linear modeling was used to analyze differences in bacterial counts.

Results: Reductions in colony-forming units (CFUs) on treated boots ranged from no detectable reduction to 0.45 $\log_{10}$ and varied by disinfectant. Percentage reductions in total bacterial counts generally were larger (albeit still modest) for AHP and QUAT disinfectants (range 37–45%) and smallest for the PHENOLIC (no detectable reduction).

Conclusions and Clinical Importance: In general, use of disinfectant footmats was associated with significant reductions in viable bacteria on overboots—albeit with variable efficacy. Footmats may be useful adjuncts to cleaning and disinfection programs for decreasing trafficking of microorganisms throughout VTHs but should not be considered as a sole prevention method.

Key words: Biosecurity; Cleaning and disinfection; Infection control.

Infection control is critical to providing high-quality patient care as well as maintaining a safe working environment for personnel in veterinary hospitals. Among 38 veterinary teaching hospitals (VTHs), 82% reported the occurrence of at least 1 epidemic of disease in patients in the previous 5 years, and 50% reported the occurrence of zoonotic disease among personnel in the previous 2 years.[1] During times of epidemic disease, it is common to find extensive environmental contamination and pathogens may persist in the environment for months after such an event.[2–5] Many North American VTHs utilize footbaths and footmats at entrances and key control points throughout the veterinary hospital in an endeavor to decrease trafficking of pathogenic microorganism on contaminated footwear. Among 31 VTHs, the most common disinfectants used in footbaths were quaternary ammonium products (42%), phenolics (39%), hypochlorite solutions (39%), and peroxygen disinfectants (19%).[6] Studies have shown that disinfectant footmats have variable efficacy for decreasing bacterial contamination of footwear or flooring and that the type of disinfectant can be critical to obtaining maximal reductions in bacterial counts.[6–10] A discussion on the spectrum of disinfectant activity is beyond the scope of this study, and thus we refer readers to a previous publication.[11] For example, mean bacterial counts from contaminated boots disinfected in peroxygen footbaths were 78% lower compared to untreated boots, but bacterial counts from contaminated boots treated with quaternary ammonium were not different than those treated with water.[6] In another study, peroxygen-containing footmats were shown to be as effective as footbaths when used upon exiting a food animal ward at a VTH.[7,10] These studies lend support to current infection control practices, but refinement of procedures is essential to providing optimal patient care.

The infection control program at Colorado State University (CSU)-VTH takes a multimodal (multiple-hurdle) approach to infectious disease control within

Abbreviations:

CSU-VTH	Colorado State University Veterinary Teaching Hospital
MAC	MacConkey agar
CFU	colony-forming unit
AHP	activated hydrogen peroxide
TSA	trypticase soy agar with 5% sheep blood

From the Department of Clinical Sciences, Colorado State University, Fort Collins, CO (Hornig, Saklou, Johnson, Malmlov, Metre, Morley, Byers); and the Department of Population Health Sciences, Virginia Tech, Blacksburg, VA (Burgess).

Corresponding author: Dr. S.R. Byers, Department of Clinical Sciences, Colorado State University, Fort Collins, CO 80523; e-mail: Stacey.Byers@colostate.edu.

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the equine and livestock hospitals including focused efforts on footwear hygiene in the form of disinfectant footbaths and footmats, as well as routine widespread environmental disinfection. For this purpose, for approximately 10 years, a peroxygen disinfectant[b] has been used in footmats and footbaths throughout these facilities. Based on prior objective investigations and our clinical experience, this use has aided in the control of infectious disease within our facility. However, peroxygen disinfectants are strong oxidizing agents, and continued use has affected the integrity of concrete and steel surfaces throughout these facilities. This damage to surfaces makes them difficult to decontaminate, providing a potential reservoir of pathogens, and can create occupational footing hazards.

Recently, a new peroxygen disinfectant, 4.25% hydrogen peroxide[a], has become available that the manufacturer reports to have broad antimicrobial activity (labeled as a broad-spectrum fungicide, virucide, and bacteriocide), and anecdotally is reportedly to be less corrosive on surfaces. We have shown that this product is comparable to other commonly used disinfectants in a directed mist application.[12] However, there currently are no published reports evaluating this product in a footmat application. The purpose of this study was to assess the efficacy of footmats containing different popular disinfectants by standardized methods to evaluate the reduction of bacterial contamination on footwear in a natural setting.

Materials and Methods

Study Overview

An experimental study was undertaken to evaluate the efficacy of disinfectant footmats for decontamination of the soles of rubber over boots[e] contaminated with organic material. Briefly, a source of contamination was created by housing an adult dairy cow for 4 days in a hospital stall. Boots were contaminated in a standardized manner by walking through this stall in a predetermined pattern for a fixed period of time. Each boot was randomly assigned to receive timed exposure to 1 of 5 treatments: no treatment (CONTROL), or exposure to 1 of 4 disinfectants by stepping on a soaked footmat (1.0% peroxygen solution;[a] VIRKON), 4.25% activated hydrogen peroxide at 1:16 dilution[b] (AHP), 1.56% (1:64 dilution) quaternary ammonium solution[c] (QUAT), and a 0.39% (1:256 dilution) phenolic solution[d] (PHENOLIC). After a 10-minute contact time, standardized areas of boot sole surfaces were sampled and cultured to quantify the reduction in total number of viable aerobic bacteria and total number of viable coliform bacteria on boots. Institutional Animal Care and Use Committee approval (IACUC protocol 11-2543A) was obtained before study initiation.

Boots

Fifteen pairs of new rubber over boots[e] were purchased for this study. Boots were numbered 1 through 30, and 4 standardized sampling zones were drawn (with a template) on each boot sole. Each sampling zone was a 20 × 1 cm rectangle along the long axis of the boot. Boots were thoroughly scrubbed with a detergent solution,[f] rinsed thoroughly, and allowed to air dry. The boots then were disinfected by soaking in 70% ethanol solution for 5 minutes and allowed to air dry. Once dried, boots were stored in new plastic bags until immediately before use.

Standardized Contamination Process

The contamination process was intended to obtain uniform bacterial contamination that was representative of typical conditions encountered in the animal housing and patient care areas of a livestock veterinary hospital. Although exact uniformity in contamination was not likely to be achieved in this process, the randomization process used helped assure that any differences in contamination were not systematically associated with treatment group assignments. For this purpose, contaminated bedding and animal waste provided the source of microbial contamination. The source of contamination was created by housing a mature dairy cow in a 3 × 5 m stall for 4 days before the start of the study. The stall was bedded with straw and not cleaned during the 4-day period, but straw was added as needed to provide adequate bedding. The cow was provided free choice grass and alfalfa hay and water and was removed from the stall immediately before the start of the study.

Boots were contaminated following a standardized process whereby an investigator would don a pair of sterilized boots and walk in a serpentine pattern for 2 minutes through the stall (timed with a stop watch), purposefully kicking through the dirty bedding and waste to enhance the potential for microbial contamination. During the contamination processes, investigators were blinded as to which treatments would be assigned to the boots they were wearing. The contaminated bedding and animal waste were turned and thoroughly mixed with a pitchfork before each contamination event. It was assumed that all boot pairs (and each right and left boot within a pair) were uniformly contaminated by this process.

Disinfectants and Footmats

Twenty liters of each disinfectant solution was prepared according to the manufacturer's directions immediately before use. The disinfectant solutions included a 1.0% VIRKON solution (6.5 ounces of powder to 20 L of water), a 4.25% AHP solution (40 ounces of concentrate to 20 L of water), a 1.56% QUAT solution (10 ounces of concentrate to 20 L of water), and a 0.39% PHENOLIC solution (2.5 ounces concentrate to 20 L of water). Four new 61 × 86 cm disinfectant footmats[g] were used for the project. Each footmat, uniquely identified by a randomly assigned letter to facilitate blinding of study personnel, was saturated (i.e., filled) with a disinfectant solution until the solution was no longer retained by the footmat (approximately 11 L). Once the footmat had been used for 6 treatment events, the disinfectant solution was replenished in the footmat (approximately 8 L).

Boot Disinfection and Sampling

Left and right boots of a pair were independently and randomly assigned to 1 of 5 treatments (CONTROL, VIRKON, AHP, QUAT, and PHENOLIC, as previously described). Personnel wearing boots then were informed of the blinded treatments (A-D) that were randomly assigned to the boots they were wearing. With boots still donned, study personnel stepped onto the footmat containing the assigned treatment (A-D) for 3 seconds, the boot was removed and then was allowed to rest (sole side up) for the timed 10-minute contact time. Boots randomly assigned to the control treatment were immediately removed and placed sole side up for a similar 10-minute waiting period.

After the requisite 10-minutes waiting period had elapsed, samples were collected from the boot sole, progressing from medial to lateral, with a sterile swab moistened with neutralizing broth,[h] which is purported to neutralize common disinfectants.[13] A single swab was used for each of the 4 zones (see subheading "Boots" above) and placed in 4 different prelabeled tubes containing

10 mL of neutralizing broth. The swabbing process was repeated for each boot in the same manner taking 6 seconds (timed) to swab each zone. Thus, 120 samples were collected from the 30 boots (15 pairs), 24 samples per treatment group. Personnel changed gloves before sampling each. Sample tubes were stored on ice before and after sample collection and were transported to the laboratory for further processing within 1 hour of collection.

Sample Processing

Sample processing began within 1 hour of collection and was completed within 6 hour of collection. All samples were stored on ice until processing. Laboratory personnel wore gloves when processing samples, which were changed between each sample. Samples were vortexed for 3 seconds and then six 10-fold dilutions were made by means of buffered peptone water (BPW)[i]. Samples (100 μL) of each dilution then were plated on tryptic soy agar plates with 5% sheep blood (TSA)[j] to quantify total aerobic bacteria and on MacConkey agar (MAC)[k] to quantify enteric bacteria, hereafter referred to as Gram-negative bacteria. No other isolate speciation was performed. All plates were incubated aerobically for 24 hours at 37°C. A follow-up count was performed at 48 and 72 hours to verify counts. All analyses were performed on 24-hour plate counts.

Data Analysis

Data were entered into a spreadsheet and validated, and descriptive statistics were calculated. The range of colony-forming units (CFUs) that allowed detection and accurate enumeration was assumed to be 25–50 CFUs,[14] and the lowest dilution for a sample which yielded plate counts in this range was used to determine the final estimated bacterial count. This final estimated CFU per cm^2 of sampled area was obtained by multiplying the number of CFUs by the dilution factor. For the purposes of analyses, samples for which the least diluted sample had <25 CFUs were considered to be below the limits of detection (enumeration) and were assigned bacterial counts of 25 CFUs, and samples with >50 CFUs in the most diluted sample were considered to be above limits of enumeration and were assigned bacterial counts of 50 CFUs for that highest dilution. Bacterial counts were transformed to $\log_{10}$ values to facilitate parametric analyses. Generalized linear modeling[l] was used to analyze differences in bacterial counts while accounting for the hierarchical nature of the data (e.g., zones on boots, boots in pairs). Bacterial colony counts ($\log_{10}$) on MAC and TSA were the outcomes of interest, each modeled separately. Disinfectant was the independent variable of interest. Least squares means for $\log_{10}$ bacterial counts were used to make comparisons among disinfectant treatments. A critical α of 0.05 was used for all statistical comparisons.

Results

In general, as a subjective assessment, the contamination process resulted in the inconsistent presence of grossly visible moisture, bedding, and fecal material on boots, despite best efforts at standardization. The adjusted (marginal) mean for control (untreated) samples was 4.5 $\log_{10}$ CFUs (95% confidence interval [CI], 4.4–4.6) for TSA plates and 3.5 $\log_{10}$ CFUs (95% CI, 3.3–3.6) for MAC plates, which is approximately 1 $\log_{10}$ CFU lower than the average for samples that used the same contamination methodology in previous studies.[6,10]

The CFUs after disinfectant exposure were below the limit of detection (<25 CFUs) for 1 of 120 (0.008%) TSA plates and 57 of 120 (47.5%) MAC plates. Within these results, 1 of 24 control samples had CFUs below limits of detection on TSA as did 4 of 24 on MAC plates, and 23 of 24 were greater than limits of quantification on TSA as were 14 of 24 on MAC. Samples that had CFU counts greater than could be accurately enumerated included 14 of 120 (11.6%) TSA and 4 of 120 (0.03%) MAC.

Overall, small but statistically significant reductions in average CFUs ($\log_{10}$) were detected after application of 2 of the 4 disinfectant solutions when evaluating total bacterial counts (TSA) and all 4 disinfectants when considering Gram-negative bacterial counts (MAC). Comparing counts from treated boots to the counts on untreated CONTROL boots, the estimated reductions varied among media type and disinfectant application (Table 1). The LS mean reduction of total bacteria (TSA) ranged from 0.08 to 0.26 logs for the 2 products that showed significant reductions (AHP and QUAT). Reductions for all 4 products ranged from 0.16 to 0.45 logs for Gram-negative bacteria (MAC); these reductions generally were largest for the QUAT and VIRKON disinfectants and smallest for the PHENOLIC disinfectant.

Discussion

Our results suggest that disinfectant footmats could be used to decrease CFUs for total bacteria and Gram-negative bacteria on the soles of overboots under conditions that simulate use in a large animal veterinary hospital. However, the amount of these reductions was modest, at best, and varied among the different types of disinfectants that were investigated. In general, the greater reductions were seen with peroxygen disinfectants (AHP and VIRKON), but neither of these treatments decreased contamination to levels that would be considered "sanitization" or "disinfection".[15] Our study and others have shown that disinfectant footmats and footbaths may be helpful, but are not absolute methods, for eliminating contamination on footwear in veterinary hospital environments.[6,10]

Although disinfectant footmats may be considered reliable in decreasing footwear contamination, the magnitude of these decreases is limited. This is perhaps not surprising given the high expectations and less than optimal conditions for their use. To achieve optimal decontamination efficacy, disinfectants should be applied to surfaces that have been thoroughly cleaned (i.e., scrubbed with detergent) and should remain in contact for sufficient time to allow optimal activity. In contrast, the intent of our study was to assess the effectiveness of disinfectant footmats as they would be used in a large animal hospital. As such, overboots were not scrubbed before disinfection and contact time was of limited duration. Thus, this use of disinfectants creates a rigorous challenge to their efficacy. Although disinfectant efficacy would likely be improved by the removal of organic debris before disinfection or increasing contact time, this evaluation emulated common practice in livestock hospitals where footmats are utilized to decrease trafficking of microorganisms on footwear as personnel move throughout the facility.

Table 1. Mean bacterial counts per cm^2 of samples obtained from rubber over boots after standardized contamination and exposure to different disinfectants in a footmat application.

	Treatment	N	LS Mean of log_{10} CFU/cm^2	95% CI	Reduction in log_{10} CFU/cm^2	95% CI	Percentage Reduction
TSA 24 hours	CONTROL	24	4.50	4.42–4.58	Ref		
	AHP	24	4.24	4.09–4.38	0.26[a]	0.10–0.43	45%
	PHENOLIC	24	4.52	4.42–4.61	−0.02[b]	−0.14–0.11	−5%
	QUAT	24	4.30	4.19–4.41	0.20[a]	0.07–0.34	37%
	VIRKON	24	4.42	4.29–4.54	0.08[a]	–0.07–0.23	17%
MAC 24 hours	CONTROL	24	3.45	3.31–3.59	Ref		
	AHP	24	3.26	3.15–3.38	0.19[a]	0.01–0.37	35%
	PHENOLIC	24	3.29	3.18–3.39	0.16[a]	0.01–0.34	31%
	QUAT	24	3.23	3.09–3.36	0.22[a]	0.03–0.41	40%
	VIRKON	24	3.00	2.99–3.01	0.45[b]	0.31–0.58	65%

MAC, MacConkey agar media; TSA, trypticase soy agar with 5% sheep blood; LS mean, least square geometric mean; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit. Different superscripts indicate a statistically significant difference ($P < .05$) in reduction between disinfectant solutions within each culture method.

Reductions in bacterial counts demonstrated in our study were notably smaller than those shown in a previous study conducted by our research group.[10] This finding possibly reflects variability in bacterial load and organic debris on overboots at the time of sample collection. Although the same standardized contamination method was used, in the current study visible contamination was less obvious than in the previous study, which correlated with the lower average bacterial counts on control boots.

Although our study focused on objective evidence of disinfectant efficacy, the impact of footmats and footbaths on decreasing risks for spread of contagious agents is probably greater than can be measured solely by bacterial counts on footwear. Footmats and footbaths can serve as visual indicators to personnel that they are entering or leaving areas of greater risk within a facility, and also can serve as a deterrent to unnecessary foot traffic, thereby decreasing the potential for spread of contamination. In this manner, footmats also may promote a culture of patient safety within veterinary hospitals, regardless of their microbiological efficacy—something that needs to be further explored and promoted in the practice of veterinary medicine. Finally, although footmats may be useful adjuncts to cleaning and disinfection programs for decreasing trafficking of microorganisms throughout VTHs, they should not be considered as a sole prevention method.

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Authorship

Study conceptualization was performed by Drs. Morley, Burgess, and Van Metre. Project supervision was provided by Drs. Morley and Byers. Dr. Burgess was responsible for laboratory procedures and was assisted by Ms. Nadia Saklou, Ms. Katlin Hornig, Dr. Valerie Johnson, and Dr. Ashlee Malmlov. Ms. Katlin Hornig was the primary author of the manuscript, assisted by Drs. Burgess, Morley, and Byers; all authors had opportunity to comment on drafts and approved the final version.

Footnotes

[a] Accel®, Virox Technologies Inc., Oakville, ON, Canada
[b] Virkon®S, Dupont, Wilmington, DE
[c] EnCompass Neutral Disinfectant Cleaner; EcoLab, St. Paul, MN
[d] Tek-Trol Disinfectant-Cleaner, Bio-Tek Industries, Inc., Atlanta, GA
[e] Tingley Rubber Corp, Piscataway, NJ
[f] Procter & Gamble CO, Cincinnati, OH
[g] Gempler's, Janesville, WI
[h] Difco Dey/Engley Neutralizing Broth, Becton Dickinson and Company, Franklin Lakes, NJ
[i] Buffered Peptone Water, Becton Dickinson and Co, Cockeysville, MD
[j] BBL Trypticase Soy Agar with 5% sheep blood, Becton Dickinson and Company, Franklin Lakes, NJ
[k] BBL MacConkey Agar, Becton Dickinson and Company, Franklin Lakes, NJ
[l] SAS v9.3, SAS Institute Inc., Carey, NC

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Conflict of Interest Declaration: Authors declare that they have not competing interests.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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