Evaluation of the efficacy of a peroxygen compound, Virkon®S, as a boot bath disinfectant

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Summary

Objective: To determine the efficacy of a 1% solution of Virkon®S as a boot bath disinfectant.

Methods: After contaminating a boot with pig manure, the examiner either stepped through a boot bath of 1% Virkon®S; stood in a boot bath of 1% Virkon®S for 2 minutes; scrubbed the boot for 30 seconds in a boot bath of 1% Virkon®S; or scrubbed the boot for 30 seconds in a water bath and then stepped through a boot bath of 1% Virkon®S. Untreated, contaminated boots, and boots scrubbed for 30 seconds with water alone, were also cultured. Five repetitions were performed for each treatment. A 75-mm^2 (0.12-sq in) area was sampled from the sole of each boot before and after treatment. Samples were diluted and cultured. Total bacterial counts per 75-mm^2 sampling area were calculated. Mean bacterial counts before and after treatments were compared.

Results: Stepping through a boot bath or standing in a boot bath of 1% Virkon®S for 2 minutes without first removing manure from boots did not disinfect boots. Disinfection was accomplished when visible manure was removed while standing and scrubbing in a boot bath of 1% Virkon®S, and when visible manure was first removed with water followed by dipping boots in 1% Virkon®S.

Implications: Removal of visible organic material from boots using a hose and (or) brush and then dipping manure-free boots in a boot bath of 1% Virkon®S provided an economical and practical alternative for effective use of boot baths on farms.

Key words: swine, boot baths, disinfection, biosecurity

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The primary risk factor for introduction of disease to a swine herd is direct exposure to infected pigs.[1,2] Another risk factor is exposure of pigs to people who may act as mechanical vectors of porcine pathogens.[1,2] Consequently, many pork production facilities require that personnel, visitors, and veterinarians disinfect their boots before entering facilities, and also when moving between groups of pigs of different ages or health status. Literature on boot bath use is scarce and usually limited to the authors' opinions on proper procedure. Quinn[3] recommended phenolic detergents for use in boot baths. He suggested that effective utilization of boot baths consisted of cleaning boots in a preliminary bath filled with dilute detergent, followed by immersion of clean boots in a second bath filled with detergent to a depth of 15 cm, for at least one minute. Quinn[3] advocated that large units prepare new boot baths daily, or when they are visibly contaminated, and that small units prepare new boot baths every 3 days. However, Quinn did not provide definitions of "large" and "small" farms.

The authors recently evaluated six types of disinfectants, including glutaraldehyde (Cidex Formula 7*, Johnson & Johnson Medical, Inc, Arlington, Texas), chlorhexidine diacetate (Nolvasan® Solution, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa), sodium hypochlorite (Clorox® , The Clorox Company, Oakland, California), povidone iodine (Betadine Solution, The Purdue Frederick Company, Norwalk, Connecticutt), a phenolic product (1Stroke Environ®, Steris Corporation, St. Louis, Missouri), and didecyl dimethyl ammonium chloride (Roccal-D Plus, Pharmacia & Upjohn Company, Kalamazoo, Michigan), utilizing various boot bath protocols.[4] We concluded that the type of disinfectant was irrelevant if organic material was not removed from the surface of boots prior to disinfection. Scrubbing was necessary to adequately remove manure. We also concluded that boot baths must be free of all organic debris, as contaminated boot baths increased boot contamination during cleaning.[4]

Moreover, in our studies, proper disinfection was accomplished only after manure-free boots were soaked in Roccal-D Plus for 5 minutes.[4] Removing all visible manure from boots and then soaking boots in a clean disinfectant bath for at least 5 minutes is not practical on most farms. Thus, some protocols were retested using a peroxygen compound, Virkon®S, (Farnam Livestock Products, Phoenix, Arizona), in an attempt to find a more appropriate disinfectant for use in boot baths.

We hypothesized that the use of Virkon®S in boot baths, if properly implemented, could assist in preventing the mechanical transmission of pathogens on footwear between groups of pigs. Our premise was that efficacious boot bath protocols should significantly reduce or eliminate the number of bacteria on the sole of the boot. The most effective protocols would result in disinfection as defined by culturing 1 viable bacterium per cm^2 of boot sole.[5]

Our study had the following four objectives: to determine whether contaminated

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boots would be disinfected by stepping through a boot bath of 1% Virkon®S, standing in a boot bath of 1% Virkon®S for 2 minutes, scrubbing the boot using a brush for 30 seconds while standing in a boot bath of 1% Virkon®S, or scrubbing the boot using a brush for 30 seconds while standing in a water bath and then stepping through a boot bath of 1% Virkon®S.

Table 1: Procedures used to determine efficacy of Virkon®S in disinfecting boots contaminated with pig manure.

Procedure	Step 1: Decontamination	Step 2: Disinfection [a]
1	None	None
2	None	Step through Virkon S
3	None	Stand in Virkon S, 2 min
4	None	Scrub in Virkon S, 30 sec [b]
5	Scrub in water, 30 sec [b]	None
6	Scrub in water, 30 sec [b]	Step through Virkon S

[a] 7.6 L of freshly prepared 1% Virkon®S solution in a rubber tub

[b] Boots scrubbed with a brush to remove gross contamination

Table 2: Post-treatment aerobic bacterial counts per 75-mm^2 (0.12-sq in) area of boot sole cultured.

Treatment*	Mean bacterial count n=5	Standard deviation
No boot bath	2.78×10^{8} [a]	6.77×10^{7}
Step through Virkon S	1.76×10^{8} [a]	6.06×10^{7}
Stand in Virkon S, 2 minutes	2.59×10^{7} [a]	1.01×10^{7}
Scrub in Virkon S, 30 seconds	20 [b]	45
Scrub in water, 30 seconds	1.04×10^{5} [c]	7.09×10^{4}
Scrub in water, 30 seconds, then step through Virkon S	120 [b]	268

[abc] Counts with different superscripts are different (P<.0001).

* Boot baths were rubber tubs containing 7.6 L of freshly prepared 1 % Virkon®S solution, or 7.58 L of water. Brushes used for scrubbing boots were dedicated to either water or Virkon®S

Materials and methods

Boots: New, size ten rubber boots (La Crosse boots, La Crosse Footwear, Inc, La Crosse, Wisconsin) that pulled over street shoes were used. Dedicated boots were used for disinfectant and for water. New boots were used for each repetition.

Boot baths: Three-gallon (11.4 L), round rubber boot baths (Fortex Rubber Hog Pan, Nasco, Fort Atkinson, Wisconsin), typically found on pork production units, were used. The baths measured approximately 43 cm (17”) in diameter and 10 cm (4”) in depth. Dedicated baths were used for disinfectant and for water.

Brushes: A long-handled nylon brush (Long-handled Gong Nylon Brush, Nasco, Fort Atkinson, Wisconsin) was used to scrub boots when indicated. The brush face measured 12.7 × 11.4 cm (5” L × 4.5” W). Nylon bristles were 4 cm (1 5/8") long. Dedicated brushes were used for disinfectant and for water.

Disinfectant: Two gallons (7.6 L) of a 1 % solution of Virkon®S were prepared according to label directions. Briefly, 2.6 ounces (73.7 g) of Virkon®S powder were added to 2 gallons (7.6 L) of water for each bath. A new bath was prepared after each single use (each time two boots were submerged in the same bath) using a clean bucket, measuring spoons, and measuring cups. The cost per 2-gallon bath was $0.81.

Experimental design: Boots were contaminated by having the examiner stand in a tub of growing-finishing pig manure for 5 to 10 seconds. After the boots had been contaminated, the examiner proceeded with one of the six procedures listed in Table 1.

Sampling and cultural examination: Five repetitions were performed for each treatment. A 75-mm^2 (0.12-sq in) area on the bottom (sole) of either the left or right boot was sampled before and after treatment using sterile cotton swabs (Hardwood Products Company LP, Guilford, Maine). Sampling sites were randomly selected from the same general area of each boot. Samples were taken immediately after the boot was treated (ie, boots were not rinsed after treatment, nor allowed to dry). Ten-mL centrifuge tubes filled with sterile water were inoculated with swab samples. Samples that could not be cultured immediately were refrigerated within 15 minutes of collection. Original samples and serial dilutions were plated onto 5% sheep blood agar and incubated aerobically for 18 to 24 hours at 37°C. Colonies of bacteria were counted and total bacterial counts per 75-mm^2 (0.12-sq in) sampling area were calculated.

Data analysis: Mean bacterial counts before and after treatments were compared using a general linear model repeated measures analysis followed by Tukey post-hoc analysis when indicated (SPSS for Windows, 8.00, 1997, SPSS Inc., Chicago, Illinois). A $\log_{10}$ +1 transformation of bacterial counts was used to stabilize the variances for statistical analysis.

Results

There were no differences among treatments in the numbers of bacteria cultured per 75-mm^2 (0.12-sq in) area of boot sole after boots were contaminated with manure. There were differences among treatments in the number of bacteria cultured post-treatment (P<.0001). There were no differences between the numbers of bacteria counted after stepping through a boot bath of 1% Virkon®S or standing in a boot bath of 1% Virkon®S for 2 minutes, compared to not using a boot bath (Table 2). The number of bacteria was less (P<.0001) when boots were either scrubbed for 30 seconds while standing in a boot bath of 1% Virkon®S, scrubbed for 30 seconds while standing in a boot bath of water, or scrubbed for 30 seconds while standing in a water bath and then stepping through a boot bath of 1% Virkon®S, compared to not using a boot bath, stepping through a boot bath of 1% Virkon®S, or standing in a boot bath of 1% Virkon®S for 2 minutes (Table 2). The number of bacteria was less ($P<.0001$) when boots were either scrubbed for 30 seconds while standing in a boot bath of 1% Virkon®S, or scrubbed for 30 seconds while standing in a water bath and then stepping through a boot bath of 1% Virkon®S, compared to scrubbing for 30 seconds while standing in a boot bath of water (Table 2). There was no difference in the number of bacteria counted after scrubbing for 30 seconds while standing in a boot bath of 1% Virkon®S compared to scrubbing for 30 seconds while standing in a water bath and then stepping through a boot bath of 1% Virkon®S (Table 2). Both of the latter protocols disinfected the 75-mm^2 (0.12-sq in) area of four of five boots sampled.

Discussion

Farm personnel use boot baths to prevent mechanical transmission of pathogens among groups of pigs. However, in the authors' experience, boot bath maintenance in most facilities is poor, and boot baths are often grossly contaminated with organic matter. People commonly avoid stepping into boot baths, or simply step through the bath without stopping to clean their boots. The results of this study confirm those of previous studies,[4] that simply stepping through or standing in a boot bath without first removing visible organic debris from boots does not provide effective boot disinfection. However, these experiments demonstrated that Virkon®S is a suitable disinfectant for use in boot baths when used appropriately. All visible organic material must be removed from the surface of the boot to achieve efficacy. After removal of organic material, Virkon®S rapidly disinfected the boot in most cases. Although there was no difference in the number of bacteria cultured when manure was scrubbed from boots while standing in a boot bath of 1% Virkon®S compared to scrubbing the manure off with water and then dipping in a boot bath of 1% Virkon®S, the latter protocol is more advantageous. The disinfectant in the boot bath will remain free of organic material and will need to be changed less often if personnel use a hose and (or) brush to remove manure from boots prior to dipping in disinfectant. Thus, producers will have decreased labor and disinfectant costs.

In conclusion, Virkon®S, when used appropriately, appears to be a convenient and cost-effective choice for a boot bath disinfectant on farms. Organic material must be removed from the boots either prior to or during disinfection with Virkon®S. If personnel choose to remove manure from boots in the bath of disinfectant, the disinfectant should be changed after each use. If organic material is removed prior to dipping in disinfectant, Virkon®S appears to rapidly disinfect boots in most cases.

Implications

- Removal of visible organic material from boots using a hose and (or) brush and then dipping clean boots in a boot bath of 1% Virkon®S provides an economical and practical alternative for effective use of boot baths on farms.
- Stepping through or standing in Virkon®S without removing organic debris first was ineffective in disinfecting boots.

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