Evaluating the efficacy of boot baths in biosecurity protocols

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Summary

Objectives: (1) To determine whether type of disinfectant, scrubbing of boots, or cleanliness of the boot bath affects boot bath efficacy; and, (2) to determine the length of time that manure-free boots must soak in disinfectant before disinfection occurs.

Methods: Boots were contaminated using pig manure, then disinfected according to individual protocols. Five repetitions were performed for each treatment. A 75-mm^2 (0.12-sq in) area was sampled from the bottom (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: The type of disinfectant was irrelevant if manure was not removed from the surface of boots prior to disinfection. Scrubbing was indicated to adequately remove manure. Contaminated boot baths increased boot contamination during cleaning. Disinfection was accomplished after manure-free boots were soaked in Roccal™-D Plus for 5 minutes.

Implications: Proper disinfection of boots includes removing all visible manure from boots and then soaking the boot in a clean bath of disinfectant for the time period recommended on the disinfectant label. Improper boot cleaning methods waste time and money and may place the herd at risk of pathogen spread.

Key words: swine, boot baths, disinfection, biosecurity

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The primary risk factor for introducing disease to a swine herd is direct exposure to infected pigs.[1,2] People, who may act as mechanical vectors to transmit porcine pathogens among groups of pigs, are believed to be another risk factor.[1,2] Consequently, most biosecurity protocols on pork production facilities require that personnel, visitors, and veterinarians disinfect their boots before entering facilities, and when moving between groups of pigs of different ages or health status.

Boot baths are often observed on pork production facilities;[3] however, there is no scientific evidence establishing the efficacy of boot bath use. Literature on boot bath use is scarce and is limited to the authors' opinions on proper procedure. Quinn[4] recommended phenolic detergents for use in boot baths. He suggested that effective use of boot baths consist of cleaning boots in a preliminary bath filled with dilute detergent, and then immersing clean boots to a depth of 15 cm (5.9 in) for at least 1 minute in a second bath filled with detergent. Quinn[4] advocated that large units prepare new boot baths daily or when visibly contaminated and small units prepare new boot baths every 3 days.

In the authors' experience, such protocols are rarely implemented on farms. Boot bath maintenance on most facilities is poor, and frequently boot baths are grossly contaminated with organic matter. People commonly avoid stepping into boot baths or simply step through the bath without stopping to clean their boots.

We hypothesized that boot disinfection could assist in preventing the mechanical transmission of pathogens on footwear between groups of pigs if properly implemented. 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 less than or equal to one viable bacterium per cm^2 of boot sole.[5]

We conducted the present study to determine:

- whether type of disinfectant affects boot bath efficacy;
- whether scrubbing versus dipping of boots affects boot bath efficacy;
- whether cleanliness of the boot bath affects efficacy; and
- the length of time that manure-free boots must soak in disinfectant before disinfection (one viable bacterium per cm^2) occurs.[5]

Materials and methods

The following supplies were used in all procedures:

- **Boots:** New size-10 rubber boots (La Crosse Footwear, Inc.; La Crosse, Wisconsin) that pulled over street shoes. Dedicated boots were used for each disinfectant. Boots were scrubbed free of manure and rinsed thoroughly between repetitions.
- **Boot baths:** Three-gallon (11 L) capacity, round rubber boot baths (Fortex Rubber Hog Pan, Nasco; Fort Atkinson, Wisconsin) typically found on pork production units were used. The baths measured 43 cm (17 in) in diameter and 10 cm (4 in) in depth. Dedicated baths were used for each disinfectant.
- **Brushes:** A long-handled nylon brush (Gong Nylon Brush, Nasco; Fort Atkinson, Wisconsin) was used to scrub boots when indicated. The brush face measured 12.7 cm (5 in) long × 11.43 cm (4.5 in) wide. Nylon bristles were 4 cm (1 5/8 in) long. Dedicated brushes were used for each disinfectant.

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- **Disinfectants:** Representatives of six classes of disinfectants were used:
  - — Aldehyde: Cidex Formula 7* (Johnson and Johnson Medical, Inc.; Arlington, Texas)
  - — Chlorhexidine: Nolvasan® Solution (Fort Dodge Laboratories, Inc.; Fort Dodge, Iowa)
  - — Chlorine-releasing agent: Chlorox® (The Chlorox Company; Oakland, California)
  - — Iodine-releasing agent: Betadine Solution (The Purdue Frederick Company; Norwalk, Connecticut)
  - — Phenol: 1Stroke Environ® (Steris Corporation; St. Louis, Missouri)
  - — Quaternary ammonium compound: Roccal™-D Plus (Pharmacia and Upjohn Company; Kalamazoo, Michigan)

Two gallons (7.58 L) of each disinfectant were prepared according to label directions (Table 1) for each bath. A new bath was prepared after each single use (each time two boots were submerged in the same bath) using a dedicated bucket, measuring spoons, and measuring cups for each disinfectant.

Procedures

The following common procedures were used:

- **Contamination:** Boots were contaminated by having the same individual stand in a tub of growing/finishing pig manure for 5–10 seconds.
- **Disinfection:** Boots were disinfected according to individual protocols. Generally, the same individual stood in the boot bath for the designated time period with boots submerged, scrubbing boot soles with brush when indicated.
- **Sampling and cultural examination:** Five repetitions were performed for each treatment. A 75-mm^2 (0.12-sq in) area was sampled from the bottom of each boot before and after treatment using sterile cotton swabs (Hardwood Products Company LP; Guilford, Maine). Fifteen-mL centrifuge tubes filled with sterile water were inoculated with swab samples and plated onto 5% sheep blood agar or refrigerated within 10 minutes of collection. Original samples and serial dilutions of original samples were plated onto 5% sheep blood agar and incubated for 18–24 hours at 37°C. Colonies of aerobic bacteria were counted and total bacterial counts per 75-mm^2 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.

Experiment One: Disinfectant type

To determine whether disinfectant type affected boot bath efficacy, the examiner stood in the boot bath for 2 minutes after boots were contaminated. All six disinfectants were tested.

Experiment Two: Scrubbing versus dipping

To determine whether scrubbing versus dipping of boots affected boot bath efficacy, after contamination the examiner either:

- stood in a phenolic boot bath (1Stroke Environ®) for 2 minutes;
- scrubbed the boot using the brush for 30 seconds while standing in a phenolic boot bath (1Stroke Environ®);
- scrubbed the boot using the brush for 30 seconds while standing in a water boot bath; or
- scrubbed the boot using the brush for 30 seconds while standing in a water boot bath and then stepped through a phenolic boot bath (1Stroke Environ®).

Experiment Three: Cleanliness of boot bath

To determine whether cleanliness of the boot bath affected efficacy, after contamination the examiner scrubbed the boot using the brush for 30 seconds while standing in a phenolic boot bath (1Stroke Environ®). The boot bath was either:

- freshly made;
- used once;
- used five times; or
- used 10 times.

Experiment Four: Disinfection time

To determine the length of time that manure-free boots must soak in disinfectant before actual disinfection (one viable bacterium per cm^2)[5] occurred, after contamination the examiner scrubbed visible manure off the boot in a water bath. The manure-free boots were then soaked in either a phenolic bath (1Stroke Environ®) or a quaternary ammonium bath (Roccal™-D Plus) for

- 1 minute,
- 5 minutes, or
- 10 minutes.

Results

Experiment One: Disinfectant type

There were no significant differences in the number of bacteria cultured per 75-mm^2 (0.12-sq in) area of boot among treatments after boots were contaminated with manure (Table 2). Standing in Cidex for 2 minutes after boots were contaminated significantly ($P < .0001$) reduced the number of bacteria cultured from the 75-mm^2 (0.12-sq in) area sampled as compared to soaking in any other disinfectant, or not using a boot bath. Standing for 2 minutes in boot baths filled with the other disinfectants tested did not significantly reduce the

Table 1: Active ingredients, dilution rate, and cost of disinfectants used

Brand Name	Active ingredient	Dilution	Cost per 2-gallon bath
Cidex Formula 7*	Gluteraldehyde	Undiluted	$55.00
Nolvasan® solution	Chlorohexidine diacetate	1 oz/gallon (29.6 mL/3.79 L)	$0.60
Chlorox®	Sodium hypochlorite	6 oz/gallon (178 mL/3.79L)	$0.20
Betadine solution	Povidone iodine	Undiluted	$35.70
1Stroke Environ®	o-pheno, o-benzyl-p-chloro-, and p-tertiary-amyl-phenols	0.5 oz/gallon (14.8 mL/3.79 L)	$0.26
Roccal™-D Plus	Didecyl dimethyl ammonium chloride	1 oz/gallon (29.6 mL/3.79 L)	$1.03

number of bacteria cultured (Table 3).

Experiment Two: Scrubbing versus dipping

There were no significant differences in the number of bacteria cultured per 75-mm^2 (0.12-sq in) area of boot sole among treatments after boots were contaminated with manure (Table 4). There was no difference between the number of bacteria counted when a boot bath was not used compared to when the person stood in a bath of 1Stroke Environ® for 2 minutes without scrubbing (Table 5). The number of bacteria was significantly less ($P<.0001$) when boots were scrubbed for 30 seconds in a bath compared to not using a bath or standing in 1Stroke Environ® for 2 minutes (Table 5). There were no significant differences in the number of bacteria counted among the three different scrubbing protocols (Table 5).

Experiment Three: Cleanliness of boot bath

There were no significant differences in the number of bacteria cultured per 75-mm^2 (0.12-sq in) area of boot sole among treatments after boots were contaminated with manure (Table 6). There was a significant difference in number of bacteria cultured post-treatment among treatments ($P < .0001$). The number of bacteria was significantly less ($P<.006$) when boots were scrubbed for 30 seconds in a bath compared to not using a boot bath (Table 7). There was no significant difference between the number of bacteria counted after scrubbing in a clean bath compared to scrubbing in a bath used once or a bath used five times (Table 7). The number of bacteria was significantly less ($P < .007$) when boots were scrubbed for 30 seconds in a clean bath compared to a boot bath used 10 times (Table 7). There was no significant difference between the number of bacteria counted after scrubbing in a boot bath used once as compared to scrubbing in a bath used five or 10 times (Table 7). There was no significant difference between the number of bacteria counted after scrubbing in a boot bath used five times compared to scrubbing in a bath used 10 times (Table 7).

Table 2: Post-contamination summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment One

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	95% confidence interval Upper bound
No boot bath	1.32×10^8	5.2×10^7	8.9×10^7	1.8×10^8
Cidex Formula 7*	1.02×10^8	1.8×10^7	5.9×10^7	1.4×10^8
1Stroke Environ®	7.78×10^7	2.4×10^7	3.5×10^7	1.2×10^8
Chlorox®	1.12×10^8	3.0×10^7	7.0×10^7	1.6×10^8
Betadine solution	6.86×10^7	2.1×10^7	2.6×10^7	1.1×10^8
Roccal™-D Plus	6.70×10^7	1.7×10^7	2.4×10^7	1.1×10^8
Nolvasan® solution	1.23×10^8	8.8×10^7	8.0×10^7	1.6×10^8

Table 3: Post-treatment summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment One

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	95% confidence interval Upper bound
No boot bath	1.36×10^{8} [a]	4.8×10^7	1.1×10^8	1.6×10^8
Cidex Formula 7*	6.79×10^{6} [b]	3.6×10^6	-2.2×10^7	3.6×10^7
1Stroke Environ®	7.96×10^{7} [a]	3.5×10^7	5.1×10^7	1.1×10^8
Chlorox®	5.30×10^{7} [a]	2.9×10^7	2.4×10^7	8.2×10^7
Betadine solution	3.98×10^{7} [a]	1.2×10^7	1.1×10^7	6.9×10^7
Roccal™-D Plus	5.54×10^{7} [a]	2.4×10^7	2.6×10^7	8.4×10^7
Nolvasan® solution	9.32×10^{7} [a]	3.9×10^7	6.4×10^7	1.2×10^8

[a,b] Different superscripts indicate statistical differences ($P<.0001$).

Table 4: Post-contamination summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment Two

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	95% confidence interval Upper bound
No boot bath	2.9×10^7	2.7×10^7	2.3×10^6	5.8×10^7
Stand in 1Stroke Environ® for 2 minutes	6.2×10^7	4.8×10^7	3.4×10^7	9.0×10^7
Scrub in 1Stroke Environ® for 30 seconds	7.7×10^7	3.1×10^7	4.9×10^7	1.0×10^8
Scrub in water for 30 seconds	1.8×10^7	1.4×10^7	-9.6×10^6	4.6×10^7
Scrub in water and step in 1Stroke Environ®	2.8×10^7	1.5×10^7	1.1×10^6	5.6×10^7

Table 5: Post-treatment summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment Two

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	Upper bound
No boot bath	5.6×10^{7} [a]	3.3×10^{7}	3.7×10^{7}	7.5×10^{7}
Stand in 1Stroke Environ® for 2 minutes	4.5×10^{7} [a]	3.0×10^{7}	2.6×10^{7}	6.4×10^{7}
Scrub in 1Stroke Environ® for 30 seconds	4.6×10^{2} [b]	278	-1.9×10^{7}	1.9×10^{7}
Scrub in water for 30 seconds	4.5×10^{4} [b]	6.0×10^{4}	-1.9×10^{7}	1.9×10^{7}
Scrub in water and step in 1Stroke Environ®	2.5×10^{5} [b]	5.6×10^{5}	-1.8×10^{7}	1.9×10^{7}

[a,b] Different superscripts indicate statistical differences ($P < .0001$)

Table 6: Post-contamination summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment Three

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	Upper bound
No boot bath	1.06×10^{8}	5.6×10^{7}	4.0×10^{7}	1.7×10^{8}
Scrubbing for 30 seconds in clean 1Stroke Environ® bath	1.33×10^{8}	6.3×10^{7}	6.7×10^{7}	2.0×10^{8}
Scrubbing for 30 seconds in 1Stroke Environ® bath used once	1.11×10^{8}	8.2×10^{7}	4.5×10^{7}	1.8×10^{8}
Scrubbing for 30 seconds in 1Stroke Environ® bath used five times	1.03×10^{8}	8.9×10^{7}	3.7×10^{7}	1.7×10^{8}
Scrubbing for 30 seconds in 1Stroke Environ® bath used 10 times	1.37×10^{8}	5.6×10^{7}	7.2×10^{7}	2.0×10^{8}

Table 7: Post-treatment summary statistics for bacterial counts per 75-mm^2 area of boot sole cultured in Experiment Three

Treatment (n=5)	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval Lower bound	Upper bound
No boot bath	9.9×10^{7} [a]	3.3×10^{7}	8.6×10^{7}	1.1×10^{8}
Scrubbing for 30 seconds in clean 1Stroke Environ® bath	822 [b, c]	1,125	-1.3×10^{7}	1.4×10^{7}
Scrubbing for 30 seconds in 1Stroke Environ® bath used once	1.2×10^{6} [b,c,d]	2.7×10^{6}	-1.2×10^{7}	1.5×10^{7}
Scrubbing for 30 seconds in 1Stroke Environ® bath used five times	9.2×10^{4} [b,c,d]	2.0×10^{5}	-1.4×10^{7}	1.4×10^{7}
Scrubbing for 30 seconds in 1Stroke Environ® bath used 10 times	2.7×10^{5} [b,d]	1.6×10^{5}	-1.3×10^{7}	1.4×10^{7}

[a–d] Different superscripts indicate statistical differences ($P<.007$)

Experiment Four: Disinfection time

There were no significant differences in the number of bacteria cultured per 75-mm^2 (0.12-sq in) area of boot sole among treatments after boots were contaminated with manure (Table 8). The number of bacteria counted among the disinfectants at different time periods did not differ significantly. The number of bacteria counted after scrubbing with water and the number of bacteria counted after using disinfectant also did not differ significantly (Table 8).

Discussion

Farm personnel use boot baths to disinfect the boot surface in an attempt to prevent the mechanical transmission of pathogens among groups of pigs. Tamási[5] reported that a surface was adequately disinfected if the count of viable bacteria did not exceed one viable bacterium per cm^2. The results of the experiments demonstrated that boot baths, as they are currently used in many pork production facilities, are not efficacious for disinfecting boots. Basic principles of proper boot bath use learned from these experiments include:

- Scrubbing visible manure from boots enhances removal of significant numbers of bacteria. Simply walking through a boot bath will not reduce bacterial counts. Standing in a boot bath without scrubbing off the manure did not significantly reduce bacterial counts except when Cidex Formula 7* was used at a cost of $55.00 per boot bath. Most units would find this cost prohibitive.
- Scrubbing visible manure off in a water bath is as efficacious as scrubbing manure off in a bath of disinfectant as far as reducing bacterial counts.

Table 8: Mean number of bacteria per 75-mm^2 area of boot sole before and after each treatment

Time sampled	Treatment group	Mean bacterial count / 75-mm^2 area	Standard deviation	95% confidence interval	
				Lower bound	Upper bound
Post contamination	1Stroke Environ® bath	2.32×10^8	1.8×10^8	1.0×10^8	3.6×10^8
	Roccal™-D Plus	1.11×10^8	3.1×10^7	-2.0×10^7	2.4×10^8
After scrubbing off manure in water	1Stroke Environ® bath	532	678	-15	1,079
	Roccal™-D Plus	536	319	-11	1,083
After 1-minute soak in disinfectant	1Stroke Environ® bath	186	303	-35	407
	Roccal™-D Plus	2	4	-219	223
After 5-minute soak in disinfectant	1Stroke Environ® bath	14	15	3	25
	Roccal™-D Plus	0	0	-11	11
After 10-minute soak in disinfectant	1Stroke Environ® bath	2	4	-1	5
	Roccal™-D Plus	0	0	-3	3

Although not tested, detergents may make manure removal easier.

- Scrubbing off manure in a clean disinfectant boot bath (1Stroke Environ®) reduces the bacterial count more than scrubbing boots in a contaminated boot bath.
- Boots that have been scrubbed free of manure and then soaked in Roccal™-D Plus for 5 or more minutes meet the standard for disinfection.[5]

Time constraints make the proper use of boot baths within production units difficult. However, spending time and money to implement boot bath procedures on a farm without using them correctly is a waste of resources. Although going through the motions of stepping in a boot bath can help increase employee awareness of biosecurity and maintain a clean workplace, this study indicates that this is an insufficient biosecurity measure, potentially placing the pigs at risk for infection because contaminated boots are being used by personnel.

Possible solutions for improved boot hygiene include:

- Use of disposable boots by visitors or others that spend short periods of time on the farm. Disposable boots are probably not durable enough for long visits.
- Providing designated boots for specific farm areas. For example, designated sets of boots would only be used in the farrowing house. These boots could be properly disinfected at the end of each day.
- Extensive procedures may be used in areas containing valuable animals, such as seedstock or in areas containing clinically ill animals. Boot stations could contain a wash area for cleaning off manure and a bath of disinfectant containing spare boots. At the boot station, personnel would remove contaminated boots, clean them, and place them in the tub of disinfectant. Then, personnel would put on the spare boots that had been soaking in the disinfectant and were ready for use. The disinfectant bath should remain relatively free of manure because only visibly clean boots will be added. Although time consuming, these methods offer the most protection against disease spread (Table 8).

In conclusion, boot stations with hoses and brushes will facilitate manure removal. Disinfectants should be selected based on efficacy, cost, ease of use, and environmental friendliness. We recommend that boot washing procedures be performed to the level of disinfection[5] if they are to be used on a farm.

Future studies will include farm audits to determine the most efficacious disinfectant based on pathogens present at the farm, stage, or room. Design of boots and boot baths that are easier to clean are planned.

Implications

- Most on-farm boot washing protocols do not disinfect boots.
- Proper disinfection of boots includes removing all visible manure from boots and then soaking the boot in a clean bath of disinfectant for the time period recommended on the disinfectant label.
- Improper boot cleaning methods waste time and money and may place the herd at risk of pathogen spread.

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