PEER REVIEWED

Efficacy of Disinfectants for Sanitizing Boots Under Dairy Farm Conditions

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Abstract

Ampicillin-resistant *Escherichia coli* (AR-*E. coli*), suspended in a manure slurry, was placed on rubber and plastic boot material surfaces to determine survival time. In addition, rubber boots were immersed in the bacteria-manure slurry, placed in water, and then several disinfectants were applied to the boots to determine the bacterial kill time. In the second phase of the study, boots were contaminated with the bacteria-manure slurry, and plastic and concrete surfaces were walked on to determine how far the bacteria could be tracked.

AR-*E. coli* was isolated from the surfaces of both rubber and plastic strips for up to one day after the strips were inoculated with in the AR-*E. coli* slurry, with the exception of rubber strips at the highest temperature tested. Alcohol and Roccal-D Plus appeared to be the most effective disinfectants used on rubber, followed by bleach. Betadine Solution, Nolvasan Solution and water showed similar efficacy. AR-*E. coli* could be isolated from boot tracks on plastic for nearly 400 ft (121.9 m) and from a concrete surface for up to 150 ft (47.7 m).

Results of this study emphasize the importance of time and temperature on the ability of disinfectants to eliminate bacterial contamination in manure and supports the use of biosecurity Good Management Practices (GMP's) to control the movement of potentially pathogenic bacteria on dairies.

Résumé

Des surfaces constituées de matériel provenant de bottes de caoutchouc ou de plastique ont été recouvertes avec du fumier liquide contenant des bactéries *Escherichia coli* résistantes à l'ampicilline (AR-E *coli*) pour déterminer le temps de survie des bactéries. Des bottes en caoutchouc ont aussi été immergées dans du fumier liquide contenant des bactéries puis dans de l'eau avant d'être désinfectées avec plusieurs produits pour déterminer le temps nécessaire à l'élimination des bactéries. Dans la seconde partie de l'étude, des bottes contaminées avec du fumier liquide contenant des bactéries ont été utilisées pour marcher sur des surfaces en plastique ou en ciment pour déterminer jusqu'à quelle distance les bactéries pouvaient être détectées.

Les AR-E *coli* ont été isolés à partir des surfaces de caoutchouc et de plastique jusqu'à un jour après immersion dans le fumier liquide à l'exception des surfaces de caoutchouc soumises à la plus haute température testée. L'alcool et le produit Roccal-D Plus semblaient les plus efficaces pour la désinfection du caoutchouc alors que l'eau de Javel l'était un peu moins. L'eau, la solution de Bétadine et la solution de Nolvasan avaient une efficacité similaire. Les AR-E *coli* pouvaient être détectés à partir des pistes produites par les bottes de caoutchouc jusqu'à une distance de 400 pieds (121.9 m) sur la surface de plastique et jusqu'à une distance de 150 pieds (47.7 m) sur la surface de ciment.

Les résultats de l'étude montrent bien l'importance du temps et de la température dans la capacité des désinfectants à éliminer la contamination bactérienne dans le fumier et supportent aussi l'utilisation des bonnes pratiques de gestion en biosécurité pour contrôler la dispersion d'agents pathogènes dans les fermes laitières.

Introduction

The dairy industry has only recently given serious consideration to biosecurity practices[1,11,12] despite their success in preventing disease outbreaks in the poultry[8] and swine industries.[5] The Foot and Mouth Disease outbreak in Great Britain during 2001 caused many dairymen to reconsider biosecurity measures to prevent introduction of new diseases into their herds. There is also a need to curtail movement of resident potential pathogens from one location on the dairy to another. In a recent study, salmonella was isolated from nearly 45% of boots worn in calving, hospital or fresh-cow pens.[9] On-dairy practices to minimize transfer of pathogens from one location to another include restricting the movement of people who work in high-risk areas, such as sick pens, fresh cow pens or calf housing areas; providing boot-wash facilities and equipment; or requiring workers to change boots and protective outer wear when moving from one area to another. While these and other Good Management Practices (GMP's) have been suggested for years, there is little published scientific data to support their adoption.

The purpose of this study was to develop information to support or refute the adoption of certain biosecurity GMP's by determining: 1) how long bacteria would remain viable on rubber and plastic surfaces at various temperatures; 2) antibacterial efficacy of commonly used disinfectants to kill bacteria when applied to the surface of rubber boots worn on dairies; and 3) how far bacteria could be tracked with boots. Bacteria in a manure slurry were applied to simulate conditions on dairies where bacterial pathogens are often protected from environmental conditions and disinfectants by organic material.

Materials and Methods

An ampicillin-resistant (AR) *Escherichia coli (E. coli)* isolated from a local dairy was used as the test bacterium to determine how long bacteria would remain viable on rubber and plastic boot material surfaces at various temperatures. Initially, the AR-*E. coli* was suspended in sterile phosphate-buffered saline (PBS) and mixed into 100 g of dairy cow manure to form a manure slurry containing 10^2 AR-*E. coli* per gram. Strips measuring 1x1 in (2.54x2.54 cm) were cut from new rubber and plastic boots. One ml of slurry was placed on each strip and spread using sterile wooden applicators. To determine viability of the bacteria, samples for culture were taken from the strips with sterile cotton swabs at 0, 10, 20 and 30 minutes; 1, 2 and 4 hours; and 1, 2, 3, 4 and 5 days after the strips were coated with the slurry. The procedure was repeated four times for both rubber and plastic at 60 °F (16 °C), 70 °F (21 °C) and 90 °F (33 °C).

For the rubber boot disinfection studies, AR-*E. coli* in sterile PBS was thoroughly mixed into 500 g of dairy cow manure to form a slurry containing 10^2 AR-*E. coli* per gram. Sufficient slurry was added to a large Pyrex dish (15x10x2 in; 38.1x25.4x5.1 cm) to permit a boot to be immersed to a depth of about 1 in (2.54 cm) to simulate stepping into manure. Each boot was left in the manure for one minute. Following removal from the manure, each manure-laden boot was placed in a glass dish (13x9x2 in; 33x22.8x5.1 cm) containing sterile water at room temperature (approximately 77 °F; 25 °C) for one minute. The water was not agitated. Finally the boot was immersed in one of the selected disinfectant solutions for one minute. Each disinfectant was tested in triplicate. After removal from the disinfectant, samples for culture were taken with sterile swabs from each boot at 2 minute intervals for a total of 30 minutes. The bottom of each boot was swabbed beginning at the toe and moving toward the arch, taking samples from each ridge to groove.

The disinfectants used in the study were bleach (5% sodium hypochlorite diluted at a rate of one ounce [oz] per gallon of water)[a], 70% isopropyl-alcohol[b], full strength Betadine Solution[c], Nolvasan Solution (1 oz per gallon)[d] and Roccal-D Plus (1 oz per gallon)[e]. Sterile water was used as a control for comparison. These disinfectants were chosen for the study because they are among those recommended by the California Department of Food and Agriculture for use as surface disinfectants.[3]

To determine the distance AR-*E. coli* could be tracked on rubber boots, a new plastic sheet taken directly from the manufacturer's package was laid over a 3 in (7.6 cm) base of sand. Measured marks were made on the plastic at 12 in (30.5 cm) intervals to indicate where to step so that a record of steps could be made. The length of each step was considered to be 2.5 ft (76 cm). A new plastic sheet was used for each test. A manure slurry containing 10^2 or 10^8 AR-*E. coli* was prepared in a glass container measuring 15x10x2 in (38.1x25.4x5.1 cm). After stepping in the slurry, an investigator wearing the manure-laden boots walked on the plastic sheet, stepping at the marked locations. Each step location was then swabbed using a sterile cotton-tipped swab. The experiment was repeated on a concrete surface.

In all trials, swabs were placed into Luria-Bertari (LB) broth and incubated at 99 °F (37 °C) for one hour. At that time, a sterile 10 ul loop of each broth culture was streaked onto an LB plate with ampicillin and incubated overnight at 99 °F (37 °C). After incubation, colonies of AR-*E. coli* were counted. The maximum count was 150 CFU/ml. Beyond this point, colonies were too numerous to count.

Results

AR-*E. coli* could be isolated from both rubber and plastic strips for up to one day after they were inoculated with the AR-*E. coli* slurry. With the exception of the rubber strips held at 90 °F (33 °C), there were no significant differences in survival time due to variation in temperature. At 90 °F (33 °C), AR-*E. coli* could not be recovered from the rubber strips after 30 minutes post-inoculation. At other time and temperature points tested, the AR-*E. coli* were recovered up to 4 hours post-inoculation, with only sporadic recovery at later times (Table 1).

There was variable efficacy among the disinfectants tested in killing the AR-*E. coli* on rubber boots exposed to manure (Table 2). Alcohol and Roccal were most efficacious, sodium hypochlorite (bleach) was intermediate, and the efficacy of Betadine and Nolvasan at the concentrations used was similar to water under the conditions of these tests.

AR-*E. coli* could be isolated from boot tracks made on plastic for nearly 400 ft (121.9 m) after stepping in

Table 1. Effect of temperature on growth of ampicillin-resistant *E. coli* on rubber and plastic surfaces inoculated with contaminated manure slurry (10^2 cfu/g AR-*E. coli*). Reported as CFU/ml +/- standard deviation.

	Rubber		
Time	60 °F (16 °C)	70 °F (21 °C)	90 °F (33 °C)
0 Min	119+/-80	119+/-80	119+/-80
10 Min	64+/-50	82+/-46	54+/-50
20Min	51+/-42	40+/-23	28+/-40
30 Min	51+/-39	38+/-21	21+/-31
1 Hr	43+/-31	47+/-26	0+/-1
2 Hr	30+/-19	36+/-19	0+/-0
4 Hr	20+/-16	28+/-14	0+/-0
1 Day	0+/-0.5	3+/-5	0+/-0
2 Days	0+/-0	0+/-0	0+/-0
3 Days	0+/-0	0+/-0	0+/-0
4 Days	0+/-0	0+/-0	0+/-0
5 Days	0+/-0	0+/-0	0+/-0

	Plastic		
Time	60 °F (16 °C)	70 °F (21 °C)	90 °F (33 °C)
0 Min	125+/-63	125+/-63	125+/-62
10 Min	79+/-42	74+/-49	94+/-50
20 Min	86+/-42	29+/-44	79+/-43
30 Min	73+/-41	53+/-38	72+/-41
1 Hr	48+/-29	42+/-31	63+/-38
2 Hr	38+/-28	33+/-21	47+/-34
4 Hr	7+/-8	28+/-20	37+/-28
1 Day	1+/-1	1+/-3	0+/-0
2 Days	0+/-0.5	0+/-3	0+/-0
3 Days	1+/-4	0+/-3	0+/-0
4 Days	0+/-0	0+/-0	0+/-0
5 Days	0+/-0	0+/-0	0+/-0

manure slurry containing 10^8 AR-*E. coli* per gram, and for 225 ft (68.6 m) when the manure slurry contained 10^2 AR-*E. coli* per gram. On the concrete surface, tracking distances were 150 ft (45.7 m) and 100 ft (30.5 m) after stepping in slurry containing 10^8 and 10^2 AR-*E. coli* per gram, respectively.

Discussion

AR-*E. coli* remained viable on rubber boot material for only 30 minutes when tested at 90 °F (33 °C), but the organism could be isolated from rubber and plastic surfaces for at least four hours at other temperatures. The effect of exceptionally low (freezing) or extremely high temperatures on bacterial viability was not tested and remains undefined.

Contaminated rubber boots were dipped in several commonly available disinfectants to determine their efficacy to either kill or reduce the number of viable bacteria. It is important to note that the use of disinfectants in this study did not strictly follow label instructions, in particular removal of organic matter and contact time. Study boots were immersed in water without agitation or scrubbing, and contact time with the various disinfectants was limited to one minute. Boots worn on dairies should be scrubbed vigorously to remove all the manure and other organic matter prior to application of disinfectants.[2,6] Ten minutes of contact time is recommended by the label for Nolvasan Solution and Roccal D-Plus, but no mention of contact time was found on the Betadine Solution label. However, on most dairies, workers do not vigorously scrub their boots, but rather spray water on the sides of the boots to reduce the manure load. In our experience, disinfectants are not routinely used on dairies to sanitize boots. If they do, contact time is usually limited to a few seconds, typically a quick dip in the solution.

The variation in efficacy of the disinfectants tested in this trial reinforces the importance of following the manufacturer's directions and recommended GMP's.[2,3,4] Several factors influence the efficacy of disinfectants, including concentration (dilution rate), temperature, pH, contamination, water quality and type of organism.[7] All of these must be considered when selecting the product for disinfecting boots on livestock operations. Alcohol, while an excellent disinfectant, has several inherent disadvantages which limit its usefulness for disinfecting boots. It can swell and harden rubber, is flammable and has a high evaporation rate.[10] Betadine and Nolvasan are effective disinfectants, however, they are compromised when organic matter is present, as observed in this study.

The amount of manure clinging to different areas of the boot sole likely influenced the variation in bacterial growth seen at different time points for each disinfectant tested. When swabbed, different areas on the boots were noted to have varying amounts of manure. This was particularly true for the areas in the deep tread of the sole. It is also probable that there were non-visible variations in bacterial populations.

Results of this study suggest that boots used on dairies potentially remain contaminated with bacteria for one day. The amount of viable bacteria on boots declines with time, however, bacteria may be transferred by boots to other locations where conditions are favorable for regrowth. If this occurs, bacteria may multiply and reach levels sufficient to cause disease. Proper disinfection of boots following use on dairy farms may reduce the spread of potentially pathogenic bacteria to distant locations on or off the dairy. Under no circumstances should boots be worn off the dairy to homes or public buildings.

In this study, bacteria were carried by boots up to 400 ft (121.9 m) from the site of original contamination, reinforcing that proper boot cleaning and disinfection practices should be used on dairies when leaving high-risk areas. Adequate water under pressure and brushes should be available to remove most of the manure from boots prior to application of disinfectants. It may take several min-

Table 2. Colony counts of ampicillin-resistant *E. coli* (CFU/ml+/- standard deviation) following application of various disinfectants to rubber boots contaminated with dairy manure.

Time (minutes)	Water	Bleach	Alcohol	Betadine	Nolvasan	Roccal
0	150+/-0	27+/-14	62+/-78	150+/-0	150+/-0	1+/-2
2	150+/-0	47+/-23	0+/-0	150+/-0	150+/-0	4+/-6
4	150+/-0	71+/-70	0+/-0	150+/-0	150+/-0	5+/-7
6	150+/-0	107+/-74	0+/-0	150+/-0	150+/-0	2+/-3
8	150+/-0	68+/-71	0+/-0	150+/-0	150+/-0	8+/-13
10	150+/-0	73+/-67	0+/-0	150+/-0	150+/-0	7+/-10
12	150+/-0	109+/-70	0+/-0	150+/-0	150+/-0	9+/-15
14	150+/-0	110+/-70	1+/-2	150+/-0	150+/-0	2+/-3
16	150+/-0	113+/-63	1+/-2	150+/-0	150+/-0	1+/-1
18	150+/-0	84+/-57	0+/-0	150+/-0	150+/-0	3+/-5
20	150+/-0	79+/-63	0+/-0	150+/-0	150+/-0	2+/-2
22	150+/-0	26+/-10	0+/-0	150+/-0	100+/-86	6+/-9
24	150+/-0	25+/-13	0+/-0	150+/-0	150+/-0	1+/-2
26	150+/-0	81+/-61	0+/-0	150+/-0	150+/-0	9+/-16
28	150+/-0	107+/-45	0+/-0	150+/-0	150+/-0	50+/-86
30	150+/-0	39+/-53	0+/-0	150+/-0	150+/-0	50+/-86

utes to clean boots highly contaminated with manure. Following thorough removal of manure, they should be disinfected using the proper concentration of disinfectant solution and allow sufficient contact time to kill the bacteria. A cleanable surface and large drain help make washing and disinfection of boots easier.

Results of these trials support the use of GMP's to control movement of potentially pathogenic bacteria on dairies. When proper cleaning and disinfection of boots is not possible, alternative strategies for preventing movement of potentially harmful bacteria can be employed. High-risk areas on the dairy should be clearly identified. Boots can be left in these high-risk areas, and other clean boots provided for use in other areas on the dairy. On large operations, workers can be restricted to high-risk locations, and not permitted to enter other areas of the dairy.

Conclusions

Routine use of biosecurity GMP's to curtail transfer of potentially harmful bacteria on dairy farms not only reduces the risk of introducing domestic pathogens onto a farm and minimizes intrafarm spread of disease, but also reduces the risk of a foreign animal disease outbreak. The adoption of these practices is essential to an effective dairy biosecurity plan.

Footnotes

[a] Clorox, Oakland, CA.

[b] 70% Isopropyl-alcohol, First Priority, Elgin, IL

[c] Betadine Solution, The Purdue Frederick Company, Norwalk, CT.

[d] Nolvasan Solution, Fort Dodge Animal Health, Overland Park, KS.

[e] Roccal-D Plus, Pharmacia Animal Health, Kalamazoo, MI.

References

1. Anon: Biosecurity practices of U.S. dairies. *Info Sheet,* USDA, APHIS, Veterinary Services, May 1999.
2. Anon: *Disinfectants: Boot disinfection.* National Biosecurity Resource Center for Animal Health Emergencies, Purdue University, www.biosecuritycenter.org.
3. Anon: *Biosecurity: Selection and use of surface disinfectants.* Animal Health and Food Safety Services, California Department of Food and Agriculture, Sacramento, CA, 2001.
4. Anon: *Biosecurity tips.* Animal Health and Food Safety Services, California Department of Food and Agriculture, Sacramento, CA, 2001.
5. Anon: *Biosecurity: Guide for pork producers.* National Pork Board, Des Moines, IA, 2002. www.porkboard.org
6. Bowman GL, Shulaw WP: Disinfection in on-farm biosecurity procedures. *Ohio State University Extension Fact Sheet* VME-0008-01. 2001.
7. Heit MC, Riviere JE: Antiseptics and disinfectants. in Adams HR (ed): *Veterinary Pharmacology and Therapeutics,* ed 8. Iowa State University, Ames, 2001, pp 783-795
8. Jeffrey JS: Biosecurity for poultry flocks. *University of California Cooperative Extension Leaflet PSFS No. 27* (www.vetmed.ucdavis.edu/vetext/INFO PO_Biosecurity.html), 1997.
9. Kirk JH, Sischo WM, Barnett SC, Collar C, Higginbotham J, Shultz T: Salmonella contamination of rubber boots worn on dairies. *Bov Pract* 36(1):11-14, 2002.
10. Rutala WA: APIC guideline for selection and use of disinfectants. *Am J Inf Control* 18(2): 99-117, 1990.
11. Sischo W: Dairy herd biosecurity. *Bovine Veterinarian* May-June: 8-9, 1997.
12. Wells SJ: Biosecurity on dairy operations: Hazards and risk. *J Dairy Sci* 83:2380-2386, 2000.