Effectiveness of using a mat filled with a peroxygen disinfectant to minimize shoe sole contamination in a veterinary hospital

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Objective—To determine the effectiveness of using a disinfectant mat filled with a peroxygen compound to prevent mechanical transmission of bacteria via contaminated footwear between the food animal ward and common breezeway of a veterinary teaching hospital.

Design—Observational study.

Sample Population—Shoe soles of individuals entering and exiting from the ward.

Procedures—A mat filled with peroxygen disinfectant was placed at the entrance to the food animal ward, and participants wiped each shoe twice on the mat surface (n = 16) or walked on the mat surface but did not wipe their shoes (17) before entering and exiting from the ward. Swab specimens were collected from the shoe soles of participants before and after mat use and submitted for bacterial culture.

Results—For both study days, as participants entered the ward, median number of aerobic bacteria isolated from shoe swab specimens collected prior to use of the disinfectant mat was not significantly different from median number isolated after use of the disinfectant mat. However, as participants exited the ward, median number of aerobic bacteria isolated from shoe swab specimens collected prior to use of the disinfectant mat was significantly higher than median number isolated after use of the disinfectant mat.

Conclusions and Clinical Relevance—Results suggest that placing a mat filled with a peroxygen disinfectant at the exit from the food animal ward of a veterinary teaching hospital may help reduce mechanical transmission of bacteria on the footwear of individuals leaving the ward. (J Am Vet Med Assoc 2006;228:1391–1396)

Infection control in the large animal wards of veterinary teaching hospitals offers unique biosecurity challenges. Large animal patients can be clinically or subclinically infected with a variety of pathogens and may be more susceptible to infection while hospitalized because of the stress of medical treatments or hospitalization itself. In addition, large numbers of staff members, students, and clients travel through the hospital and contact patients on a daily basis, increasing the risk of transmission of infectious organisms.

Nosocomial Salmonella infections are of particular concern in the large animal wards of veterinary teaching hospitals, with multiple outbreaks of salmonellosis involving patients at large animal hospitals having been reported. In 2000, an outbreak of salmonellosis at the Purdue University Large Animal Veterinary Teaching Hospital resulted in closure of the hospital for nearly 10 weeks, causing lost instructional time for students, relocation of senior students to other teaching hospitals, negative publicity, and lost hospital income.

Many veterinary teaching hospitals have implemented biosecurity programs to prevent the spread of Salmonella spp and other pathogens throughout the hospital. An important component of such biosecurity programs is controlling the mechanical transmission of pathogens by staff members and students working in the hospital through the strategic placement of boot baths and disinfectant mats throughout the hospital. Boot bath effectiveness has been tested under simulated conditions with cattle and swine manure and pathogens. In 2 studies, investigators stepped in swine manure to contaminate rubber boots and then used various procedures to clean the boots. Scrubbing visible contamination from the boots for 30 seconds followed by disinfection with adequate contact time was necessary to achieve a significant reduction in bacterial counts. In both studies, however, disinfectant on swab samples was diluted in sterile water but not chemically inactivated; therefore, residual disinfectant could have led to false-negative results. In a third study, plastic boots contaminated with porcine reproductive and respiratory syndrome virus and swine manure were immersed for 5 seconds in a boot bath of undiluted 6% sodium hypochlorite. The number of boots contaminated with viral RNA was significantly reduced following sodium hypochlorite treatment, compared with immersion in water, but the sodium hypochlorite was not inactivated after samples were collected, so the disinfectant was in contact with the sample from the time of collection until laboratory testing occurred. Moreover, the failure to inactivate the sodium hypochlorite could have interfered with testing, resulting in false-negative results.

To our knowledge, only a single study has evaluated the effectiveness of boot baths in reducing bacterial contamination of footwear in a veterinary teaching hospital and contact patients on a daily basis, increasing the risk of transmission of infectious organisms.
hospital. Rubber boots contaminated by walking through manure and bedding in a stall that housed a mature bull were treated by immersion for 2 seconds in a boot bath containing either a quaternary ammonium or peroxygen disinfectant. Mean bacterial counts from boots treated with the quaternary ammonium disinfectant did not differ from counts for untreated control boots. Although mean bacterial counts were significantly lower for boots treated with the peroxygen disinfectant than for control boots, the treated boots were not considered sterilized.1

By contrast, how effectively disinfectant mats prevent transmission of bacterial pathogens has not been determined. The purpose of the study reported here, therefore, was to determine the effectiveness of using a disinf ectant mat filled with a peroxygen compound to prevent mechanical transmission of bacteria via contaminated footwear between the food animal ward and common breezeway of a veterinary teaching hospital. Specific objectives were to determine whether the number of aerobic bacteria on footwear decreased following use of the disinfectant mat during entry to or exit from the food animal ward, whether bacteria would accumulate in the disinf ectant mat after repeated use, and the economics involved in disinf ectant mat maintenance.

Material s and Methods
Study design—The study was conducted at the Purdue University Veterinary Teaching Hospital during a period of normal hospital operation. On April 21, 2005, and again on April 28, 2005, swab samples were collected from the footwear of the first 20 students, staff members, and clinicians who entered the single food animal ward at the teaching hospital between 6:00 and 11:00 AM and exited again during that time period. For each individual who participated in the study, a swab sample was collected before and after the person used a disinf ectant mat placed at the entrance to the ward when entering the ward and again when exiting the ward. Additional samples were collected from individuals who made repeated trips to the food animal ward during the time of the study, but results for only the first set of samples collected were included in data analyses.

The study was designed to be able to detect a difference of 105,466 cfu between samples collected before and after use of the disinf ectant mat with 90% power, assuming the SD for number of bacteria in swab samples was 100,000 cfu. During the time of the study, the veterinary teaching hospital did not have regulations specifying the type of footwear that had to be worn in the hospital. Therefore, samples were collected from a variety of footwear, including rubber boots, plastic boots, and street shoes. For purposes of the present study, all types of footwear are referred to as shoes.

During both days of the study, a 71 × 36-inch disinf ectant mat1 was placed at the entrance to the food animal ward and filled with 10 gallons of a 1% solution of a peroxygen disinfectant. The volume of 10 gallons was selected because preliminary testing revealed that this volume was required for the disinf ectant to cover the soles of the shoes of people who stepped on the mat. During the first day of the study (April 21, 2005), individuals participating in the study were asked to step on the mat and wipe their shoes on the mat surface a total of 2 times/shoe before entering or exiting the food animal ward. During the second day of the study (April 28, 2005), individuals participating in the study were asked to walk on the mat surface so that each shoe contacted the mat once, but to not wipe their shoes on the mat, before entering or exiting the food animal ward. Time needed to fill the disinf ectant mat prior to use and to empty and clean it after use was measured. This study was not considered human subject research by the institutional review board.

Sample collection—For individuals participating in the study, swab samples were collected from the soles of the shoes before and after the individual used the disinf ectant mat. To minimize variances in sample collection technique, all swab samples were collected by a single individual (SFA). To facilitate sample collection, other investigators (MA, JLS) aseptically transferred supplies to and from the investigator collecting the swab samples (SFA). Investigators (SFA, MA, JLS) donned masks, nitrile gloves, plastic boots, and clean outerwear during sample collection and handling, and donned masks, nitrile gloves, and clean lab coats during bacterial culture to prevent sample contamination. Protective gear was donned prior to the start of sample collection on each date and was not changed between individual sample collections on a single date unless the protective gear was breached.

As participants entered the food animal ward, swab samples were collected from the heel area of the shoe sole. As participants exited the food animal ward, swab samples were collected from the area of the shoe sole under the ball of the foot. A coin toss was used to determine whether the right or left shoe was swabbed prior to use of the disinf ectant mat; the contralateral shoe was then swabbed after use of the disinf ectant mat. Thus, if the right shoe was randomly selected to be swabbed before use of the disinf ectant mat, then the left shoe was swabbed after use of the mat, and vice versa. For collection of swab samples, chairs were placed within 2 feet of either side of the disinf ectant mat, and participants sat in the chairs for collection of samples prior to use of the mat.

On the first study day, participants wiped each shoe twice on the mat and then immediately held up the shoe to be swabbed while standing on the edge of the mat with the other foot. On the second study day, participants stepped off the mat, and samples were collected within 1 foot of the disinf ectant mat.

Sterile stainless steel washers were used to standardize the area swabbed on each shoe to 6.16 cm2. For collection of each swab sample, a sterile washer was placed on the sole of the shoe, and a sterile polyester-tipped applicator swab was used to swab the area designated by the hole in the washer. The swab was then immediately placed in a tube containing 2 mL of Dey-Engley broth2 to inactivate residual disinf ectant. Tubes containing samples were maintained in coolers supplied with cold packs until all samples were collected. Samples were then transported to the laboratory for processing.

A 1-mL sample of disinf ectant was aseptically collected from the mat prior to the start of each day of the study and after each participant exited the food animal ward. Thus, a total of 21 samples was collected on each study day. A pipet with individual sterile tips was used to collect these samples. Each sample was immediately placed in a tube containing 1 mL of Dey-Engley broth2 to inactivate the disinf ectant. Tubes containing samples were maintained in coolers supplied with cold packs until all samples were collected. Samples were then transported to the laboratory for processing.

Laboratory processing—All samples were processed within 5 hours after the last sample was collected. All original tubes containing swab samples in 2 mL of Dey-Engley broth and original tubes containing 1 mL of disinf ectant mat solution in 1 mL of Dey-Engley broth were mixed by hand agitation. A 100-μL aliquot of each original sample was plated on 5% sheep blood agar.3 Additionally, 3 (April 21) or 4 (April 28) serial 10-fold dilutions were made with sterile Dey-Engley broth, and a 100-μL aliquot of each dilution was plated on 5% sheep blood agar. All samples were incubated
aerobically at 36.9°C for 24 hours, and number of cfu was counted. If individual colonies could not be discerned, number of aerobic bacteria was recorded as too numerous to count. A sample set was defined as the set of blood agar plates representing all dilutions for a single sample. If possible, the plate within each sample set that had between 30 and 300 cfu was used for estimating total number of aerobic bacteria, after accounting for dilution factors, for that sample. In some cases, no plates within a given sample set had between 30 and 300 cfu. Sample sets in which all plates had < 30 cfu were assigned a value of 30, and bacterial count was estimated by adjusting for the appropriate volume and dilution. Similarly, sample sets in which all plates had > 300 cfu were assigned a value of 300, and bacterial count was estimated by adjusting for the appropriate volume and dilution. Estimated total number of aerobic bacteria was divided by 6.16 to calculate number of aerobic bacteria/cm² of shoe sole.

For both days of the study, bacterial culture of samples of mat disinfectant collected prior to use of the mat did not yield any growth. Therefore, bacterial count for these samples was recorded as 600 cfu/mL, as this was the limit of detection for the technique.

Following initial bacterial culture procedures, all swab and disinfectant samples in Dey-Engley broth collected on the first day of the study were shipped overnight on cold packs to the Iowa State University Veterinary Diagnostic Laboratory for isolation of Salmonella spp. Samples arrived at the Veterinary Diagnostic Laboratory the morning after collection and were processed immediately. Each sample was plated on brilliant green with novobiocin agar and xylose-lysine-tergitol 4 agar, and plates were incubated at 37°C. The remaining volume of each sample and the original collection swab, if present, were transferred to a tube containing 9 mL of tetrathionate broth and tubes were incubated at 42°C.

Samples in tetrathionate broth were subcultured after 24 and 48 hours of incubation on brilliant green with novobiocin agar and xylose-lysine-tergitol 4 agar. For delayed enrichment, the tetrathionate broth was left at room temperature for 5 days, and a 1-mL aliquot was transferred to a second tube containing 9 mL of tetrathionate broth and tubes were incubated at 42°C. All plates were examined for suspect colonies after 24, 48, and 72 hours of incubation. Suspect colonies were subcultured to Kligler’s iron agar to test for hydrogen sulfide production and lysine iron agar to test for lysine.

Data analysis—For swab sample data, the Wilcoxon matched-pairs signed rank test was used to compare median cfu per cm² of sole at entry to and exit from the ward and to compare median cfu per cm² of sole before and after use of the disinfectant mat. The null hypothesis was that the median number of CFU/cm² of sole did not change after use of the disinfectant mat. The Mann-Whitney test was used to compare estimated number of CFU/cm² of sole.

Results

Hospital patient data—During both study days, the food animal ward was populated with inpatients during sample collection. On the first study day (April 21, 2005), the ward housed 3 adult cattle and 4 calves. On the second study day (April 28, 2005), the ward housed 2 adult cattle and a single calf. On each study day, one of the calves housed in the ward had a fever of unknown origin, but the remaining patients reportedly did not have signs of any gastrointestinal tract disorders. Outpatients were not present in the ward on either day during sample collection.

Participants—During the first study day, swab samples were collected from 16 individuals. Four individuals entered and exited the ward twice during the sample collection period, and data from the second set of samples collected from these individuals were not included in analyses. One individual wiped his shoes a total of 6 times instead of 4 (ie, twice for each shoe), but data for this individual were included.

During the second study day, swab samples were collected from 17 individuals. Three individuals entered and exited the ward twice during the sample collection period, and data from the second set of samples collected from these individuals were not included in analyses. One individual stepped on the mat twice with each shoe, and another individual stopped and shuffled his feet while standing on the mat. Data from both of these individuals were included.

Bacterial culture of shoe swab specimens—Salmonella organisms were not isolated from any shoe swab specimens or from any disinfectant samples collected on the first day of the study. As participants entered and exited the food animal ward, median number of aerobic bacteria isolated from shoe swab specimens collected prior to use of the disinfectant mat was not significantly different (P = 0.029 for first day of study, and P = 0.041 for second day of study) from median number isolated after use of the disinfectant mat (Table 1). However, as participants exited the ward, median number of aerobic bacteria isolated from shoe swab specimens collected prior to use of the disinfectant mat was significantly higher than median number isolated after use of the disinfectant mat.

On the first day of the study, median number of aerobic bacteria isolated from shoe swab specimens collected prior to use of the disinfectant mat when participants were exiting the ward was significantly higher than median number isolated prior to use of the disinfectant mat when participants were entering the ward. On the second day of the study, median numbers of aerobic bacteria isolated prior to use of the disinfectant when participants were entering and exiting the ward were significantly different (P = 0.073). For both days, median number of aerobic bacteria isolated after use of the disinfectant mat when participants were entering the ward was not significantly different (P = 0.029) higher than median number isolated prior to use of the disinfectant mat when participants were entering the ward.

On the first day of the study, for samples collected as participants entered the ward, 14 of 16 samples collected prior to use of the disinfectant mat and all 16 samples collected after use of the disinfectant mat met the criterion for disinfection (ie, ≤ 10³ cfu/cm²). For samples collected as participants exited the ward, 6 of 16 samples collected prior to use of the disinfectant mat and 15 of 16 samples collected after use of the disinfectant met this criterion.
On the second day of the study, for samples collected as participants entered the ward, 13 of 17 samples collected prior to use of the disinfectant mat and 14 of 17 samples collected after use of the disinfectant mat met the criterion for disinfection. For samples collected as participants exited the ward, 8 of 17 samples collected prior to use of the disinfectant mat and 14 of 17 samples collected after use of the disinfectant mat met the criterion for disinfection.

Bacterial culture of mat disinfectant samples—On the first day of the study, median bacterial count for disinfectant solution obtained from the mat after each of the first 10 participants used the mat was 600 cfu/mL (range, 600 to 10,200 cfu/mL). Median bacterial count for disinfectant solution obtained after each of the second 10 participants used the mat was 600 cfu/mL (range, 600 to 54,400 cfu/mL), which was significantly (P = 0.017) higher than median count for samples collected after the first 10 participants.

On the second day of the study, median bacterial count for disinfectant solution obtained from the mat after each of the first 10 participants used the mat was 600 cfu/mL (range, 600 to 10,200 cfu/mL). Median bacterial count for disinfectant solution obtained after each of the second 10 participants used the mat was 600 cfu/mL (range, 600 to 640 cfu/mL), which was not significantly (P > 0.999) different from median count for samples collected after the first 10 participants.

Economics of disinfectant mat use—The fixed cost to purchase 2 disinfectant mats (ie, the number needed to span the distance across the entryway to the food animal ward) was estimated as $378. Variable costs associated with disinfectant mat use included labor costs and cost of the disinfectant. Members of the hospital staff took 10 minutes to prepare disinfectant solution for and fill a single mat, and 12 minutes to empty and clean a single mat. Thus, labor costs to maintain both mats were estimated at $7.92/d if both mats were filled and cleaned once a day. The cost for disinfectant solution needed to fill both mats once a day was $9.00/d; therefore, total variable costs were estimated at $16.92/d.

Discussion

Results of the present study suggest that placing a mat filled with a peroxygen disinfectant at the exit from the food animal ward of a veterinary teaching hospital may help reduce mechanical transmission of bacteria on the footwear of individuals leaving the ward, in that median bacterial counts on the shoes of individuals exiting the ward were significantly lower after use of the disinfectant mat, compared with median count immediately before use of the mat. In contrast, we did not detect a significant difference in median bacterial counts before and after mat use as participants entered the food animal ward. This could have been a function of the low statistical power of the study or the relative cleanliness of shoe soles as participants entered the ward.

Importantly, although median bacterial count was decreased by use of the disinfectant mat as participants exited from the food animal ward, the shoe soles of participants were not sterile. Similarly, previous studies have found that cleaning procedures rarely resulted in sterilization of footwear. In the only other published study on footwear disinfection performed in a veterinary teaching hospital, for instance, contaminated boots were immersed in a disinfectant solution for 2 seconds, and 7 minutes later, samples were taken for bacterial culture. However, even after 7 minutes of contact time with the disinfectant, boots were not considered sterilized. Thus, disinfectant mats should be considered, at most, as only 1 part of an overall infection control program.

Use of a practical benchmark is desirable when evaluating efficacy of hospital protocols. The benchmark used in the present study was the standard for disinfection proposed by Bohm (ie, ≤ 10 cfu/cm²). On the first study day, only 6 of 16 swab specimens collected prior to use of the disinfectant mat as partici-
pants exited the food animal ward met this benchmark, whereas 15 of 16 specimens collected after use of the mat did. Similarly, on the second study day, only 8 of 17 swab specimens collected prior to use of the disinfectant mat as participants exited the food animal ward met this benchmark, whereas 14 of 17 specimens collected after use of the mat did. However, it must be understood that this benchmark is somewhat arbitrary. A more useful benchmark could be whether number of cfu of a designated target organism (such as Salmonella spp) was less than the estimated infectious dose for that organism.

In the present study, when participants were asked to wipe each shoe on the mat twice, median bacterial count in the disinfectant solution in the mat was significant higher after the second 10 mat uses than after the first 10 mat uses. Other studies12 have reported similar findings when contaminated footwear was placed directly into a boot bath. However, when personnel simply walked across the disinfectant mat, median bacterial count in the disinfectant solution after the second 10 uses was not significantly different from median count after the first 10 uses. Thus, bacterial contamination of the disinfectant solution was greater when the disinfectant mat was used aggressively, presumably because wiping shoes on the mat physically removed more organic material than simply walking on the mat without wiping. Accumulation of bacteria in mat disinfectant solution requires further study to determine whether pathogens survive in the mat solution, whether the mat solution can be the source of an infectious dose of a pathogen, and the frequency with which the disinfectant solution in a mat would have to be changed to minimize pathogen contamination.

There were several important limitations to the present study. First, the study was conducted on 2 separate days, and the number of cattle housed as inpatients in the food animal ward during sample collection varied between study days. Thus, effectiveness of the 2 methods of using the disinfectant mat (wiping shoe soles vs simply walking across the mat) cannot be directly compared. Further studies are needed to determine if one method is superior to the other. Second, the study did not investigate the effects of disinfectant mat use on viral contamination. Bacteria were used as a marker because bacteriologic culture is considered a test. Third, Salmonella organisms were not isolated from any of the samples. Thus, we were unable to test the effectiveness of using the disinfectant mat to prevent mechanical transmission of Salmonella spp. Fourth, the statistical power to detect significant differences in bacterial count was low because of the small sample size (sample size was limited to the number of personnel entering and exiting the food animal ward on 2 given mornings) and high variability in bacterial counts. Fifth, we collected swab specimens from the heel area of the shoe sole as participants entered the ward and swab specimens from the ball area of the shoe sole as participants exited the ward, and we took specimens from the left versus right shoe before and after mat use. However, we did not test whether people accumulate the same quantity of bacteria on these 2 areas of the shoe sole or whether the left and right shoes of a single individual were equally contaminated, potentially introducing some bias. Finally, bacterial counts used in the present study were only estimates. For the present study, serial dilutions of the samples were created and plated on separate agar plates. Bacterial count was determined by counting bacterial colonies on plates that had between 30 and 300 cfu. However, if none of the plates for a particular sample had > 30 cfu, a value of 30 cfu was assigned to the undiluted sample (appropriate calculations were then performed to determine cfu/cm² or cfu/mL), and if none of the plates for a particular sample had < 300 cfu, a value of 300 cfu was assigned to the highest dilution (again, appropriate calculations were then performed to determine cfu/cm² or cfu/mL). For the 64 shoe swab specimens collected on the first study day, 30 did not yield colony counts > 30 cfu, and 5 did not yield colony counts < 300 cfu. For the 21 disinfectant samples collected on this day, 12 did not yield colony counts > 30 cfu. For the 68 shoe swab specimens collected on the second study day, 26 did not yield colony counts > 30 cfu. For the 21 disinfectant samples collected on this day, 19 did not yield colony counts > 30 cfu. Thus, use of assigned values for plate colony counts potentially increased median values, and calculated values were likely overestimates of the true bacterial counts, limiting our ability to detect significant difference between groups.

We calculated that at Purdue University, the cost of maintaining 2 disinfectant mats at the entrance to the food animal ward was approximately $16.92/d, or $6,176/y, if the disinfectant solution in the mats was changed only once a day. In deciding whether to implement the use of disinfectant mats, Purdue University personnel considered economics as well as compliance with use of the mat. In the present study, compliance was undoubtedly good because 3 investigators were coaching and assisting each participant as he or she used the mat. However, despite this individual attention, some participants still did not follow instructions. Another important factor to be considered is that people traffic among hospital areas is not the sole form of mechanical transmission of pathogens. Animals and equipment (eg, carts and portable x-ray units) are also moved throughout the hospital. Because some animals might refuse to step on the disinfectant mats and many pieces of equipment cannot be rolled across the mat, the costs associated with emptying, moving, replacing, and refilling the mats to allow movement of animals and equipment must be considered. As a result, to date, Purdue University has not adopted use of disinfectant mats as a part of its biosecurity procedures.

c. Difco D/E neutralizing broth, Becton, Dickinson & Co, Sparks, Md.
d. BD BBL stacker plate, trypticase soy agar with 5% sheep blood, Becton, Dickinson & Co. Sparks, Md.
e. Brilliant green with novobichin agar, Becton, Dickinson & Co, Sparks, Md.
f. Xylose lysine tergitol 4 agar, Becton, Dickinson & Co, Sparks, Md.
Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Evaluation of the prevalence and onset of lung lesions and their impact on growth of lambs

Joseph A. Daniel et al

Objective—To determine the prevalence and temporal onset of lung lesions in lambs and the impact of lung lesions on growth of affected lambs.

Animals—259 crossbred wether lambs from a single flock in the upper Midwestern United States.

Procedures—An observational study was conducted. Lambs born in the spring and fall were slaughtered at a finished weight or at a predetermined time point. Lungs of each lamb were examined and classified as normal, moderate lesions (consolidation > 5% but ≤ 50% of any lobe), or severe lesions (consolidation > 50% of any lobe). Data were examined to detect effects of prevalence or severity of lung lesions on growth and carcass traits.

Results—57 of 89 (64%) spring-born lambs had lung lesions characterized by consolidation of lung tissue. A small number of lambs had pulmonary adhesions or active abscesses. In contrast, only 31 of 108 (29%) fall-born lambs had lung lesions. Severe lung lesions were associated with a significant reduction in average daily gain. Severe lung lesions were not detected until the middle of the finishing period and were associated with culture of Mannheimia haemolytica or Pasteurella multocida.

Conclusions and Clinical Relevance—Analysis of results indicates that the prevalence of severe lung lesions can be quite high in lambs. Severe lung lesions can lead to greatly decreased growth performance of lambs. (Am J Vet Res 2006;67:890–894)