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Evaluation of the effects of footwear hygiene protocols on nonspecific bacterial contamination of floor surfaces in an equine hospital

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EQUINE

Objective—To evaluate the effects of footwear hygiene protocols on bacterial contamination of floor surfaces in an equine hospital.

Design—Field trial.

Procedures—Footwear hygiene protocols evaluated included use of rubber overboots with footbaths and footmats containing a quaternary ammonium disinfectant, rubber overboots with footbaths and footmats containing a peroxygen disinfectant, and no restrictions on footwear type but mandatory use of footbaths and footmats containing a peroxygen disinfectant. Nonspecific aerobic bacterial counts were determined via 2 procedures for sample collection and bacterial enumeration (contact plates vs swabbing combined with use of spread plates), and the effects of each footwear hygiene protocol were compared.

Results—There were no consistent findings suggesting that any of the protocols were associated with differences in numbers of bacteria recovered from floor surfaces. Although there were detectable differences in numbers of bacteria recovered in association with different footwear hygiene protocols, differences in least square mean bacterial counts did not appear to be clinically relevant (ie, were < 1 $\log_{10}$).

Conclusions and Clinical Relevance—Although cleaning and disinfection of footwear are important aids in reducing the risk of nosocomial transmission of infectious agents in veterinary hospitals, the numbers of aerobic bacteria recovered from floor surfaces were not affected by use of rubber overboots or the types of disinfectant used in this study. Further study is warranted to evaluate the usefulness of footwear hygiene practices relative to their efficacy for reducing transmission of specific pathogens or decreasing nosocomial disease risk. (*J Am Vet Med Assoc* 2006;228:1068–1073)

Abbreviations

CSU-VMC	Colorado State University Veterinary Medical Center
QAC	Quaternary ammonium compound
CCU	Critical care unit

Maintaining high standards of hygiene is essential to the mission of providing quality care at any medical facility, including mitigation of the risk of nosocomial infection. Nosocomial infections are caused by in-hospital exposure to pathogens via contact with infected patients or contaminated hands, surfaces, instruments, aerosols, or liquids. Thus, understanding and controlling microbial contamination of materials that come into contact with patients are important in control of nosocomial infections. However, because few studies have been published in which efficacy of infection control and hygiene practices were evaluated, many of these practices are widely employed from a first-principles approach in the hope that any effort is better than no effort. This includes the use of various footwear hygiene protocols despite uncertainty about the efficacy of their impact on nosocomial infection risk.[1]

Although methods for evaluating footwear hygiene are not well defined, many investigators[1-3] have evaluated the bottoms of boots and compared bacterial counts on contaminated surfaces with those on disinfected surfaces. Such methods may constitute a practical way of comparing effects of different disinfectants on the boots themselves, but they do not necessarily reflect the activity of pathogens in the environment. The efficacy of footbaths as determined by decreased bacterial counts on footwear in experiments that mimic conditions in swine and cattle production environments has been studied.[2-5] Those studies have revealed that pathogens can be recovered from footwear worn in animal production environments even after disinfection. However, conditions encountered in animal production environments are not perfectly relevant to conditions encountered in veterinary hospitals. This is partly because of differences in the quantity of feces and organic material encountered in the different facilities and partly because of differences in the relative importance of microbial contamination in hospitals versus production environments. Another study[6] has focused on in vitro evaluations of disinfectant efficacy. Multiple investigations[1,2,4,7,8] have revealed that exposure to disinfectants under ideal conditions substantially reduces microbial numbers, and some have involved evaluations of footwear or experimental surfaces under conditions intended to mimic those

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encountered in the field. Although these studies were useful in evaluating theoretical efficacy, results may not be directly applicable to the efficacy of disinfectants when applied in practical conditions.

Footbaths and footmats containing disinfectant are commonly used to control nosocomial infections. Results of a survey[1] of veterinary teaching hospitals in the United States and Canada indicated that disinfectant footbaths or footmats were used in 30 of 31 hospitals even when their practical impact on infection control was questioned. Although use of disinfectant footbaths decreases microbial contamination of rubber overboots, the choice of disinfectants and the conditions in which they are used are critically important.[1,4] To be practically useful, disinfectants must be able to kill microbes in the presence of organic material but must also be nontoxic to humans and other animals, safe in the environment, economical, and not damaging to the clothing or surfaces to which they are applied.[9,10] Other relevant concerns are that disinfectants have rapid action, the temperature range at which they maintain efficacy is practical, and they have residual activity on various surfaces. These issues all impact the practicality and ease of use of disinfectants in footbaths and are thus important for acceptance of footwear hygiene protocols by personnel because full compliance is less likely if surface disinfectants require 15 to 30 minutes of contact time before achieving an effective degree of sanitation. Additionally, experience at CSU-VMC and other facilities has revealed that increasing the convenience of use with disinfectant footmats (vs footbaths) can dramatically improve compliance.[1]

Although it has been proposed that reductions in microbial numbers on the surface of footwear should be associated with reduced risks of nosocomial infections, field trials in which footbaths reduced microbial numbers in the environment or reduced numbers of specific pathogens have not been published and there are few studies in which a decrease in infectious disease risk in association with use of footwear hygiene practices was reported.[11-13] The purpose of the study reported here was to evaluate the effect of 3 footwear hygiene protocols on bacterial contamination of floor surfaces in an equine hospital.

Materials and Methods

The floor of the equine ward at the CSU-VMC was sampled after implementation of 3 footwear hygiene protocols. Nonspecific aerobic bacterial counts were used to compare the effects of each protocol. The 2 procedures used for sample collection and enumeration of bacterial counts were also compared.

Design—Rigorous hygiene and biosecurity protocols were in place at the CSU-VMC prior to initiation of the study. For the Equine Ward, those protocols included the requirement that all personnel wear hospital-dedicated rubber overboots in the inpatient housing and management areas. Personnel were required to walk through (briefly stepping into and out of) footbaths and footmats whenever they were encountered and to clean footwear whenever they became visibly soiled. Boots were donned and removed in a designated primary staging area near the entrance upon entering or exiting the inpatient areas. Rubber overboots were not required when personnel worked in the outpatient receiving area or the attached exam and procedure rooms (**Figure 1**). At the initiation of the study, all footbaths contained a QAC as a disinfectant.[a] These footwear hygiene procedures had been used in the Equine Ward for approximately 6 years prior to initiation of the study. Samples were first obtained from floor surfaces at designated locations throughout the Equine Ward, sampling was repeated the following week, and the second footwear hygiene protocol was initiated.

For the second hygiene protocol, the QAC disinfectant in the footbaths and footmats was replaced with a peroxygen disinfectant[b] containing potassium peroxymonosulfate; use of rubber overboots was still required. After 6 weeks, environmental samples were obtained from the same locations as were sampled after the first protocol and sampling was repeated during the following week. This second protocol was used for a total of 10 weeks prior to initiating a third protocol that eliminated restrictions on footwear worn in the Equine Ward, although mandatory use of footbaths and footmats containing the peroxygen disinfectant was continued. Environmental samples were obtained from the same locations after 9 weeks of the third protocol, and sampling was repeated the next week.

The 2 disinfectants evaluated were selected on the basis of their use in footbaths and footmats at the CSU-VMC prior to the study. There was no specified length of time required for contact of boots with footmats or for immersion of footwear in footbaths. Although there were no specific requirements for postapplication treatment of rubber overboots, in most circumstances, disinfectant solutions were not rinsed from footwear after application. It was common for personnel to use footbaths and footmats frequently and repeatedly as they moved throughout the hospital environment.

There were a few exceptions to the general study conditions. During all 3 phases of the study, personnel entering the CCU were required to wear area-dedicated rubber overboots supplied by the CSU-VMC; these boots were donned and removed in the CCU staging area. Footbaths and footmats in the CCU area were filled with the peroxygen disinfectant throughout all 3 phases of the study. Also, throughout the study period, footbaths outside of stalls in the CCU were

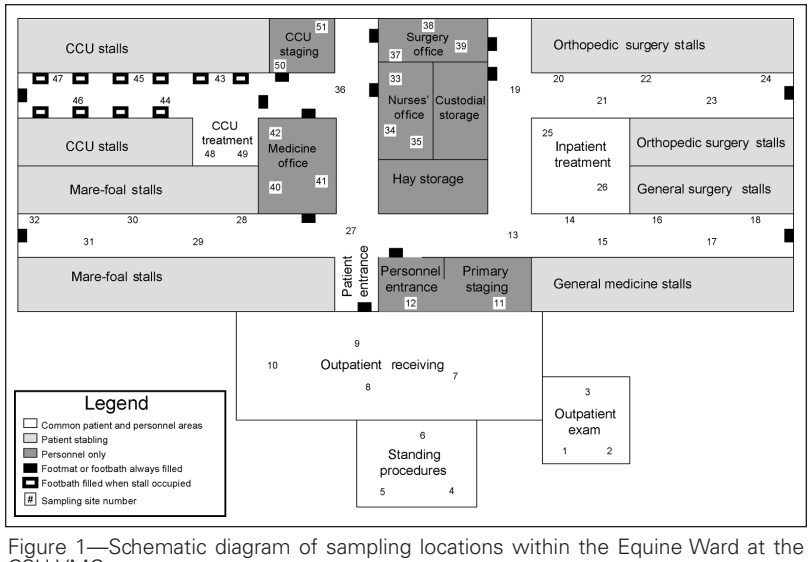

Figure 1—Schematic diagram of sampling locations within the Equine Ward at the CSU-VMC.

filled only when the corresponding stalls were occupied. Slightly different management procedures were used in the CCU area because that area is used to stable patients requiring frequent observation as a result of their clinical status and because those patients were at increased risk of shedding *Salmonella enterica*. However, animals that were confirmed to be shedding *S enterica*, *Streptococcus equi* subsp *equi*, *Clostridium* spp associated with enterocolitis, or other contagious pathogens were housed in an isolation unit in another building. Numbers of horses admitted to the equine hospital were obtained from medical records in the 3 weeks during which samples were obtained and also in the month prior to each of the 3 sampling periods.

Sample site selection—Fifty-one sites representing high-traffic areas were selected for sampling within the Equine Ward (Figure 1). Samples were collected from each site in the morning on all 6 sampling dates. Sampling sites were grouped into 5 categories: outpatient areas (sites 1 to 10), general inpatient aisles (sites 11 to 24, 27 to 32, and 36), inpatient personnel areas (sites 25, 26, 33 to 35, and 37 to 42), the CCU aisle (sites 43 to 47), and CCU personnel areas (sites 48 to 51). Outpatient areas included the outpatient reception area and rooms used for standing procedures (eg, endoscopy) and for examinations and outpatient treatments. General inpatient aisles included all sites adjacent to stalls in 3 aisles where inpatients were housed, inpatient treatment areas, sites in connecting corridors, and sites near the personnel entrance to the inpatient areas. Inpatient personnel areas included sites in the nurses' office, medicine service office, and surgery service office. The CCU aisle included sites adjacent to stalls in the area where CCU patients were housed. The CCU personnel areas included the staging area used for changing into attire dedicated for use in the area and a portion of the CCU treatment area dedicated to storage of supplies, medications, and medical records. Areas used primarily for patient housing (ie, stalls) and areas immediately adjacent to drains were excluded.

Floor surfaces and drains—Floors in the Equine Ward of the CSU-VMC are constructed of concrete, with the exception of the outpatient receiving area, which is composed of asphalt (Figure 1). Concrete floors were first poured in 1978 and have undergone repair in several places since then. The asphalt floor in the outpatient receiving area was installed in 1994. The concrete floors were of different surface types, including unsealed smooth concrete, unsealed rough concrete, and sealed smooth concrete. In general, floors in areas restricted to personnel use had the smoothest finish and were sealed. Other concrete surfaces (ie, where horses were walked) had a rougher surface texture. Drains in the inpatient areas were primarily interspersed in the aisles between rows of stalls. There were no drains in the outpatient receiving area, but there were centrally located drains in the attached rooms.

Footmats, footbaths, and rubber overboots—Disinfectant footmats[c] were used in 8 locations (ie, at personnel and patient entrances and outside of doorways to the nurses' office, medicine service office, surgery service office, and custodial storage area; Figure 1). Footmats were constructed of a foam core covered with synthetic mesh on the top surface and water-impervious cloth on the side and bottom surfaces. Throughout the study, footmats were routinely filled with disinfectant solution at approximately 8:00 AM, at 5:00 PM after rinsing thoroughly with water from a hose, and anytime they were observed to be drying out. Between the first and second protocols, footmats were rinsed with copious volumes of water to remove residual QAC disinfectant, allowed to dry overnight, and then refilled with the peroxygen disinfectant. Disinfectant footbaths[d] were used at all other locations. Footbaths were routinely emptied and refilled with disinfectant solution to a depth of approximately 6 inches at about 8:00 AM and 5:00 PM daily. Footbaths were also emptied and refilled whenever the solution was visibly contaminated with debris or drying out. Footbaths in the CCU area outside of the doors to patient stalls were filled only when the adjacent stall was occupied. Although bacterial contamination of footbaths and footmats and concentrations of disinfectant solutions were not evaluated as part of the study, routine periodic evaluation of disinfectant solution concentrations at the CSU-VMC has revealed that disinfectant solutions maintain > 90% of initial concentration under conditions used in this hospital when changed as described. One style and brand of rubber overboots[e] was recommended and used by nearly all personnel working in the Equine Ward.

Cleaning and disinfection—Routine cleaning of the Equine Ward was performed daily. Soiled bedding and fecal material were placed in dumpsters dedicated for use in this area and wheeled through the doors at the end of the aisles. When stalls were vacated, bedding material was removed and the stall was rinsed with copious volumes of water. Stalls were scrubbed with a detergent,[f] disinfected with hypochlorite solution[g] and allowed at least 15 minutes of contact time; rinsed thoroughly with water; and disinfected again with QAC disinfectant[a] according to manufacturer's directions, with at least 20 minutes of contact time. Cleaning solutions were rinsed out of stalls into drains in adjacent aisles. After cleaning and disinfecting stalls, hot water and steam were applied to stall surfaces and aisles by use of a heated pressure washer.[h] In addition, aisles in the inpatient portions of the hospital were also swept and hosed every morning. Floors in inpatient personnel areas were swept daily and disinfected with a QAC solution diluted according to the manufacturer's instructions. The outpatient receiving area was hosed with water twice weekly, and QAC disinfectant was applied twice monthly. Floors of the outpatient exam and treatment areas that were adjacent to outpatient receiving were cleaned and disinfected nightly with water, detergent, and QAC solution. Ambient temperature in the Equine Ward was maintained between approximately 18.3° to 23.9°C (65° to 75°F) during the study period.

Sample collection—Samples were collected via 2 procedures. In the first procedure, two 15 × 60-mm contact plates (ie, agar plates with a convex surface that protrudes above the plastic container edge) were used at each site: 1 MacConkey agar plate[i] and 1 blood agar plate.[j] Contact plates were pressed gently against floor surfaces for approximately 5 seconds. After sample collection, plates were transported to the lab and incubated under aerobic conditions for 18 hours at 35°C. In the second procedure, a sterile cotton-tipped swab soaked in 1 mL of Dey-Engley neutralizing broth[k] was used to sample a 100-cm^2 area immediately adjacent to the site sampled with the contact plate. Each swab was rubbed across the floor in a zigzag pattern in 3 different directions covering the entire area within the 100-cm^2 template. Each swab was then placed into neutralizing broth and briefly mixed, and 20 µL of the inoculated neutralizing broth was immediately transferred into 180 µL of saline (NaCl) solution at a pH of 7.4. Serial dilutions of saline solution at concentrations of 10^{-2} to 10^{-8} were made in a 96-well plate, and 10-µL aliquots of each dilution were plated onto one quarter of a 100-mm MacConkey and a blood agar plate (total, 4 dilutions/plate). Plates were incubated aerobically for 18 hours at 35°C.

Control samples—Positive and negative control samples were used to estimate the maximum amount of bacterial recovery. For negative control samples, a sterile swab was dipped into the neutralizing broth and subsequently diluted

and plated, as described. For the positive controls, *Escherichia coli*[l] was incubated overnight in trypticase soy broth[m] to a concentration of 1.93×10^9. Two hundred microliters of the *E coli* culture was spread over three 100-cm^2 templates on a concrete surface that had previously been cleaned with 70% ethanol. Two other templates served as background controls, which provided a count of microbes on the floor after cleaning. Positive control and background control samples were collected with sterile cotton swabs and processed as described. Plates were incubated for 18 hours at 35°C.

Enumeration and limits of quantification—Blood agar plates were used to quantify numbers of aerobic gram-positive and aerobic gram-negative bacteria, whereas MacConkey agar was used for quantification of aerobic gram-negative bacteria that were primarily of enteric origin. It was not possible to use culture methods that would allow recovery of every type of pathogenic bacteria because many bacterial species require different enrichments or environmental conditions for growth. It was considered that evaluation of the numbers of bacteria recovered by use of the culture techniques employed would yield data that could be generalized to apply to various potentially pathogenic bacterial species.

Bacterial CFUs were enumerated on contact plates, and counts from the surface area of the plates (28.27 cm^2) were converted to values for a surface area of 100 cm^2 to facilitate analysis. As recommended by the manufacturer of the contact plates, only CFU counts in the range of 20 to 200 per plate (71 to 700 CFUs/100 cm^2) were considered valid for enumeration. Counts of < 20 CFUs/plate were considered below limits of valid enumeration for this sampling and recovery method and were arbitrarily assigned a value of 71 CFUs/100 cm^2 for statistical analyses. Counts > 200 CFUs/plate were considered above the limits of valid enumeration for this sampling and recovery method and were arbitrarily assigned a value of 750 CFUs/100 cm^2 for statistical analyses. For spread plates, diluted samples that yielded 6 to 75 colonies/10 µL of inocula were considered valid for enumeration, which, at the lowest dilution evaluated, was equivalent to 1,000 CFUs/100 cm^2. Samples that yielded < 6 CFUs/10 µL of inocula were assigned a value of 1,000 CFUs/100 cm^2 for statistical analyses. No samples yielded > 75 CFUs/10 µL of inocula at the highest dilution cultured; thus, all were within valid limits of enumeration.

Statistical analysis—Bacterial concentrations were transformed to $\log_{10}$ values to allow parametric data analyses. Regression analysis for mixed effect models[n] was used to analyze differences in bacterial numbers. The $\log_{10}$ bacterial numbers were used as the dependent variable, and the independent variables of interest were footwear hygiene protocol (overboots and QAC vs overboots and peroxygen vs peroxygen and no footwear restrictions) and the area from which samples were obtained (outpatient areas, general inpatient aisles, inpatient personnel areas, CCU aisle, and CCU personnel areas). The sampling date, recorded categorically as the first through the sixth date, was included in regression models as a random effect. Least square mean values for $\log_{10}$ bacterial concentrations and 95% confidence intervals were determined from these models and used to compare differences associated with the experimental treatments by use of the Tukey-Kramer method of correction for multiple comparisons. Statistical comparisons were evaluated in a protected fashion, ensuring that the overall or type-3 effect was significant before evaluating the pairwise differences for least square mean values. A critical value of $\alpha = 0.05$ was used for all statistical evaluations.

Results

Many samples yielded growth that was either above or below the limits of valid enumeration (**Table 1**). As expected, estimated bacterial counts were higher when culture was performed on blood agar, compared with

Table 1—Summary of enumeration of bacterial growth (No. [%]) yielded by culture of samples from floor surfaces in various areas of the Equine Ward at the CSU-VMC. In each category, 306 samples were cultured.

	MacConkey agar spread plates	MacConkey agar contact plates	Blood agar spread plates	Blood agar contact plates
Above enumeration limits	0	24 (7.8)	0	151 (49.3)
Within enumeration limits	34 (11.1)	84 (27.5)	201 (65.7)	110 (36.0)
Below enumeration limits	272 (88.9)	198 (64.7)	105 (34.3)	45 (14.7)

Table 2—Least square (LS) mean $\log_{10}$ bacterial counts recovered from floor surfaces at 51 sites in the Equine Ward at the CSU-VMC associated with 3 footwear hygiene protocols. Bacterial counts are expressed as CFUs/100 cm^2 transformed into $\log_{10}$ values.

	MacConkey agar contact plates		MacConkey agar spread plates		Blood agar contact plates		Blood agar spread plates	
Variable	**LS mean**	**(95% CI)**	**LS mean**	**(95% CI)**	**LS mean**	**(95% CI)**	**LS mean**	**(95% CI)**
Footwear hygiene protocol								
Overboots and QAC	2.003[z]	(1.965–2.097)	3.247[y]	(3.100–3.394)	2.283[y]	(2.163–2.402)	3.763[z]	(3.386–4.140)
Overboots and peroxygen	2.084[z]	(2.018–2.151)	3.094[y,z]	(2.993–3.195)	2.619[z]	(2.512–2.727)	3.791[z]	(3.443–4.140)
Peroxygen	2.025[z]	(1.965–2.086)	3.052[z]	(2.966–3.138)	2.625[z]	(2.517–2.733)	3.632[z]	(3.294–3.971)
Sampling site								
CCU aisle	2.255[a]	(2.144–2.365)	3.234[a]	(3.111–3.357)	2.702[a]	(2.583–2.821)	3.933[a]	(3.539–4.326)
General inpatient aisles	2.122[a]	(2.070–2.173)	3.141[a,b]	(3.066–3.216)	2.697[a]	(2.623–2.770)	3.923[a]	(3.688–4.158)
Outpatient	1.897[a,b]	(1.819–1.975)	3.074[b]	(2.979–3.169)	2.514[b]	(2.421–2.606)	3.926[a]	(3.624–4.228)
CCU personnel areas	2.082[a]	(1.959–2.206)	3.153[a,b]	(3.018–3.287)	2.433[b]	(2.302–2.563)	3.543[a,b]	(3.111–3.974)
Inpatient personnel areas	1.879[b]	(1.796–1.961)	3.054[b]	(2.955–3.152)	2.200[c]	(2.104–2.295)	3.322[b]	(3.008–3.635)

Within a variable category, differences between values with different superscripts were significant ($P < 0.05$). Differences in footwear hygiene protocols and sample locations in the Equine Ward were controlled via multivariable analyses.
95% CI = 95% Confidence interval.

counts obtained with MacConkey agar. For MacConkey agar, 11.1% of samples yielded growth that could reliably be enumerated on spread plates, whereas 27.5% could reliably be enumerated when contact plates were used. In contrast, more samples had growth on blood agar that fell within countable limits on spread plates (65.7%), compared with contact plates (36%). Although there were detectable differences for bacteria recovered under different footwear hygiene protocols (**Table 2**), differences in least square mean values for recoverable bacteria were small ($< 1 \log_{10}$) and may not be clinically relevant. Additionally, there were no consistent trends suggesting that footwear hygiene protocols created systematic differences in bacterial numbers on floor surfaces. There were, however, differences among least square mean values, suggesting that the type of use may have influenced bacterial contamination of floor surfaces. Areas with access limited to personnel had lower least square mean bacterial counts than those that were accessed by both personnel and patients. In addition, areas in the CCU had higher bacterial counts than other inpatient areas. Outpatient areas had the lowest least square mean bacterial counts.

Numbers of horses admitted to the equine hospital were similar in the 3 weeks during which samples were collected and also in the month prior to each of the 3 sampling periods. Fifty-eight, 46, and 57 horses were admitted during each of the 3 sampling periods, respectively, and 217, 185, and 188 horses were admitted during the month prior to each of those sampling periods, respectively.

Discussion

Despite widespread implementation of footwear hygiene protocols in large animal hospitals with the assumption that microbial transmission will be reduced,[1] there were no consistent trends in the present study suggesting that the footwear hygiene protocols evaluated were associated with systematic differences in numbers of aerobic bacteria recovered from floor surfaces. Although differences in numbers of bacteria recovered were detected when different footwear hygiene protocols were used, differences in least square mean bacterial counts may not have been clinically relevant. Although these findings suggested that bacterial contamination of floor surfaces in equine hospitals was not affected by use of rubber overboots or the type of disinfectant used in footbaths, this does not diminish the importance of cleaning and disinfection of footwear. The design of the present study did not permit evaluation of the efficacy of footwear hygiene practices for reducing transmission of pathogens, and further study is warranted to more specifically evaluate the efficacy of footwear hygiene for decreasing nosocomial disease risk.

Previous work[1] has revealed that the use of disinfectant footbaths decreases bacterial contamination on the soles of the type of rubber boots that were used in the present study and that there are differences in the short-term disinfection efficacy of the same 2 disinfectants as were evaluated in this study. Interestingly, these differences in decontamination effects for footwear did not appear to impact bacterial contamination on floor surfaces. Assuming that the differences in estimates of bacterial counts on the surfaces of boot soles were accurate,[1] there are multiple possible explanations for the lack of effect on bacterial contamination of floor surfaces. It is possible that footbath and footmat use was too infrequent to counteract the influence of contact with sources of bacterial contamination such as feces and contaminated bedding. It is also possible that personnel footwear plays a relatively minor role in contamination and spread of bacteria on floor surfaces, a possibility that is supported by the observed differences in bacterial numbers recovered from sites in various areas of the equine hospital. In general, areas dedicated for use by personnel had lower bacterial counts than did areas where equine patients had contact with surfaces, and there were generally lower bacterial counts in outpatient areas, compared with general inpatient areas, which had generally lower bacterial counts than did CCU areas (Table 2). Given that the differences in bacterial counts were generally small, it is also possible that the relatively rigorous cleaning and hygiene efforts overcame relative deficits that might be caused by differences in footwear hygiene protocols. It is also possible that differences in the short-term efficacy of disinfectants for footwear decontamination[1] do not correlate with the effects that are realized after more extended contact periods and that, in this regard, the disinfectants have similar efficacy. The different footwear hygiene protocols may thus be approximately equivalent in efficacy without regard to their absolute efficacy (ie, they could be equally effective or equally ineffective).

There are few studies[11-13] in which a decrease in infectious disease risk in association with use of footwear hygiene practices was reported. The risk of *Campylobacter* spp infections in commercial broiler flocks in Great Britain was significantly reduced by the application of effective hygiene barriers, including appropriate use of disinfectant boot dips.[11,12] Similarly, results of an epidemiologic study of *Campylobacter* spp infection in broiler flocks in The Netherlands indicated that there was decreased risk of infection when separate boots were used for each broiler house and when disinfectant footbaths were used for personnel entering the broiler houses.[13] Although such data support the idea that footbaths are effective in reducing the risk of bacterial infections in environments of intensive animal housing, no studies have been published that report similar efficacy for those measures in veterinary hospitals.

In the present study, 2 sampling and culture methodologies were used to compare effectiveness and practicality for general use. Sampling with contact plates required less preparation and processing time, but counts were subject to upper limits of valid enumeration, which was not a limitation when swabs and spread plates were used. Bacterial counts obtained by use of the swab and spread plate methods were generally higher than counts obtained with the contact plates, a finding that may be attributable to sampling of a larger surface area. It is also possible that least square mean bacterial counts on contact plates were affected

by truncation of low and high values at the limits of valid enumeration. Additionally, the swab sampling method was better suited to uneven or corrugated surfaces when compared with sample collection with contact plates, an effect that may have accounted for some of the differences in least square mean bacterial counts. Although differences in least square mean values were small, those counts were influenced by the use of truncated values for samples that yielded growth above or below limits for valid enumeration, which would have tended to minimize true differences.

The culture conditions used in this study did not allow for recovery of all bacteria found on floor surfaces and, importantly, did not account for bacteria that are adapted to anaerobic and microaerophilic conditions. However, procedures for sampling and culture were uniform for all samples and allowed for unbiased comparisons of bacterial concentrations derived from the different protocol periods. The study was not designed to evaluate the risk for transmission of specific pathogens, such as *Salmonella* spp, *Staphylococcus aureus*, *Streptococcus* spp, and *Actinobacillus* spp, but the effects of disinfection and cleaning protocols should have the same general effect on common bacterial pathogens as they did on bacteria that were recovered by use of the study methodology.

It was unavoidable that personnel were aware of changes in disinfection procedures in the Equine Ward during the study period, and it is therefore possible that knowledge of changes and the study objectives among staff could have altered behavior and thus biased the outcome. However, although changes in procedures were widely advertised, it was not publicized that the effects of these changes were being studied. Further, personnel were not informed of details regarding sites where samples were to be collected or the schedule for sampling, and samples were collected twice during each study period at wide intervals. Therefore, it was considered unlikely that personnel awareness of study conditions affected results.

a. A-464-N, Airkem Professional Products, Ecolab Corp, Saint Paul, Minn.
b. 1% Virkon S, Antec International, a DuPont Co, Sudbury, Suffolk, UK.
c. Disinfection entrance mat, 34 × 24 × 1 inch, Gempler's, Madison, Wis.
d. Mix-A-Tub heavy-duty black plastic tub, No. RG177, 7-gallon capacity, Argee Corp, Santee, Calif.
e. Rubber work boots, style 1400, Tingley Rubber Corp, South Plainfield, NJ.
f. Tide with bleach, Procter & Gamble Corp, Cincinnati, Ohio.
g. Clorox bleach, 1:32 dilution, The Clorox Co, Oakland, Calif.
h. HS-3000, Landa Water Cleaning Systems, Camas, Wash.
i. BD BBL MacConkey agar, Becton-Dickinson, Franklin Lakes, NJ.
j. BD BBL trypticase soy agar with 5% sheep red cells, Becton-Dickinson, Franklin Lakes, NJ.
k. Difco D/E Broth, Becton-Dickinson, Franklin Lakes, NJ.
l. ATCC strain number 25922, American Type Culture Collection, Manassas, Va.
m. BD BBL trypticase soy broth, Becton-Dickinson, Franklin Lakes, NJ.
n. PROC MIXED, SAS, version 9.1, SAS Corp, Cary, NC.

References

1. Morley PS, Morris N, Hyatt DR, et al. Evaluation of the efficacy of disinfectant footbaths as used in veterinary hospitals. *J Am Vet Med Assoc* 2005;226:2053–2058.
2. Kirk J, Boggs C, Jefferey J, et al. Efficacy of disinfectants for sanitizing boots under dairy farm conditions. *Bovine Pract* 2003;37:50–53.
3. Amass S, Vyverberg B, Ragland D, et al. Evaluating the efficacy of boot baths in biosecurity protocols. *J Swine Health Prod* 2003;8:169–173.
4. Amass S, Ragland D, Spicer P. Evaluation of the efficacy of a peroxygen compound, Virkon S, as a boot bath disinfectant. *J Swine Health Prod* 2001;9:121–123.
5. Kirk J, Sischo W, Barnett S, et al. *Salmonella* contamination of rubber boots worn on dairies. *Bovine Pract* 2002;36:11–14.
6. Gasparini R, Pozzi T, Magnelli R, et al. Evaluation of in-vitro efficacy of the disinfectant Virkon. *Eur J Epidemiol* 1995;11:193–197.
7. Antec International Web site. DuPont animal health solutions—Virkon S. Available at: www.antecint.co.uk/main/virkons.htm. Accessed May 6, 2005.
8. Ewart S, Schott H, Robinson R, et al. Identification of sources of *Salmonella* organisms in a veterinary hospital and evaluation of the effects of disinfectants on detection of *Salmonella* organisms on surface material. *J Am Vet Med Assoc* 2001;218:1145–1151.
9. Morley PS. Biosecurity of veterinary practices. *Vet Clin North Am Food Anim Pract* 2002;18:133–155.
10. Langsrud S, Moretro T, Sundheim G. Characterization of *Serratia marcescens* surviving in disinfecting footbaths. *J Appl Microbiol* 2003;95:186–195.
11. Evans SJ, Sayers AR. A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. *Prev Vet Med* 2000;46:209–223.
12. Gibbens JC, Pascoe SJ, Evans SJ, et al. A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Prev Vet Med* 2001;48:85–99.
13. Van de Giessen AW, Bloemberg BPM, Ritmeester WS, et al. Epidemiological study on risk factors and risk reducing measures for *Campylobacter* infections in Dutch broiler flocks. *Epidemiol Infect* 1996;117:245–250.