Isolation of *Salmonella* spp from the environment of dairies without any history of clinical salmonellosis

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**Objective**—To determine whether *Salmonella* spp could be isolated from the environment of free stall dairies in Wisconsin without any history of clinical salmonellosis and determine the serotype and antimicrobial susceptibility of any *Salmonella* isolates recovered from the environment.

**Design**—Cross-sectional study.

**Study Population**—20 free stall dairies with no history of clinical salmonellosis.

**Procedures**—Dairy owners completed a questionnaire regarding management and production practices. Multiple swab samples were obtained from the free stall facilities and submitted for bacterial culture for *Salmonella* spp. Odds ratios were calculated to compare herd-level risk factors between dairies from which *Salmonella* organisms were isolated and herds from which *Salmonella* organisms were not isolated.

**Results**—*Salmonella* organisms were isolated from 9 of the 20 (45%) dairies. *Salmonella* serotype Meleagridis was isolated from 4 dairies, *S* Meleagridis and *S* Kentucky were isolated from 2 dairies, *S* Meleagridis and *S* Cyprus were isolated from 1 dairy, *S* Cerro was isolated from 1 dairy, and *S* Corvallis was isolated from 1 dairy. All isolates were susceptible to all antimicrobial agents tested. None of the potential risk factors analyzed demonstrated a significant association with an increased likelihood of isolating *Salmonella* spp.

**Conclusions and Clinical Relevance**—Environmental *Salmonella* contamination was demonstrated on free stall dairies with no history of clinical salmonellosis. (J Am Vet Med Assoc 2004;225:574–577)

Numerous studies have examined the prevalence with which *Salmonella* organisms can be isolated from the feces of dairy cattle and the serotype distribution and antimicrobial susceptibility patterns of strains that have been isolated. Other studies have examined farm- and herd-level risk factors associated with fecal shedding of *Salmonella* spp in dairy cattle. Increasing concerns have been leveled at the dairy industry with respect to its potential role in promoting antimicrobial resistance of *Salmonella* spp. Of particular concern are the multiple-drug resistance patterns demonstrated by some *Salmonella* serotype

Newport and 5 *Typhimurium* isolates obtained from the feces of dairy cattle in areas of intensive dairy production.

During clinical investigations of large dairies experiencing outbreaks of salmonellosis, we have occasionally collected environmental swab samples from multiple sites throughout a facility and been surprised at the number of samples from which *Salmonella* organisms were isolated. However, there is little information regarding the background level of environmental *Salmonella* contamination on free stall dairies, even though efforts at controlling and preventing clinical salmonellosis would likely benefit from greater data concerning those sites and animal populations from which organisms are more likely to be obtained. This becomes even more important in light of the difficulties encountered in developing safe and effective vaccines for salmonellosis and the increasing regulatory awareness of, and concern about, antimicrobial use in food-producing animals.

In this light, the purposes of the study reported here were to determine whether *Salmonella* spp could be isolated from the environment of free stall dairies in Wisconsin without any history of clinical salmonellosis and determine the serotype and antimicrobial susceptibility of any *Salmonella* isolates recovered from the environment. We also wanted to determine whether there were herd-level factors associated with the identification of environmental *Salmonella* contamination. The study was intentionally confined to free stall dairies with average to above-average production for the predominant breed of dairy cattle on the premises. This intentional preselection bias toward herds with superior production using the most contemporary diary herd housing system, and one that has become increasingly popular, was a deliberate attempt to examine environmental salmonellosis in what the authors perceive to be the future direction for dairy herd management systems.

**Materials and Methods**

**Herd selection criteria**—Bovine practitioners in the state of Wisconsin were contacted and asked to suggest dairies that employed a free stall housing design, were currently milking > 200 cows, and had not had any confirmed cases or clinical history suggestive of enteric salmonellosis during the past 12 months. Owners of the herds identified by these practitioners were contacted by telephone and asked to participate in the study; owners were advised that they would be required to complete an in-person interview and questionnaire regarding details of their production and manage-
multifactorial analysis practices. Only herds with above-average production for the predominant breed of adult cattle on the farm were selected for inclusion in the study.

Collection of environmental swab samples—Multiple environmental swab samples were obtained from each farm included in the study. Briefly, cotton-tipped swabs moistened in buffered peptone water (pH, 7.2) were dragged manually along flooring surfaces throughout the facility until saturated with fecal material. Samples were obtained from the flooring in every pen in which lactating cows, nonlactating cows, and heifers in the late stages of pregnancy were housed and from the flooring of all common alleyways, parlor holding areas, and walkways. Samples were also obtained in a similar manner from equipment used for manure handling, specifically mechanical scrapers and skid loaders. Floor surfaces were sampled after automated mechanical scraping or manure removal equipment had serviced each location. None of the study farms used a flush system. Feed bunk and water trough surfaces were not sampled.

Swabs were placed in a sterile screw-cap tube containing 20 mL of buffered peptone water and kept on ice until transferred to an incubator; all samples were transferred to an incubator between 3 and 4 hours after collection. Samples were collected throughout the year, with samples from 7 herds collected during the summer (July, August, and September), samples from 3 herds collected in the fall (October, November, and December), samples from 5 herds collected in the winter (January, February, and March), and samples from the remaining 5 herds collected in the spring (April, May, and June).

Microbiologic procedures—Samples were incubated with loose caps at 35°C overnight and then subcultured in selenite and tetrathionate selective-enrichment broths. For the tetrathionate broth, 0.2 mL of iodine was added just before addition of the sample. For both the selenite and tetrathionate broths, approximately 100 to 200 μL of sample was added. Selenite and tetrathionate broths were incubated overnight at 35°C and then subcultured on brilliant green agar and XLT4 agar plates. Culture plates were examined after 24 and 48 hours of incubation for colonies with typical Salmonella colony morphology. Suspect colonies were identified biochemically as Salmonella spp by means of a commercial system. All Salmonella isolates were serotyped by means of the slide agglutination method incorporating commercially available antisera and by means of the Spicer-Edwardstube agglutination method.

Antimicrobial susceptibility testing—Salmonella isolates were tested for susceptibility to amikacin, amoxicillin-clavulanic acid, ampicillin, cephalothin, cefazolin, gentamicin, tetracycline, ticarcillin, and trimethoprim-sulfamethoxazole by means of an automated antimicrobial susceptibility system and for susceptibility to ceftiofur and florfenicol by means of the antimicrobial disk diffusion method, as described by the National Committee for Clinical Laboratory Standards. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, E coli ATCC 35218, and Enterobacter faecalis ATCC 29212 were used as control strains.

Statistical analyses—Potential risk factors for isolation of environmental Salmonella spp were tested by means of methods for pair-matched data and analyzed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). Commercial software was used for all calculations. Risk factors that were examined included herd size (greater than or less than median herd size), production (greater than or less than median rolling herd average), manure handling system in the free stall facility (mechanical scraper or slatted floor), whether manure was spread on fields used for forage, whether an animal protein source was incorporated in feed rations for lactating cows, whether milking was performed 2 or 3 times a day, whether common-use equipment was involved in manure handling and feed distribution, and whether the facility was constructed all at once or had been constructed in 2 or more phases. The Fisher exact test was used to test for associations between risk factors and identification of environmental Salmonella contamination; values of P < 0.05 were considered significant. To examine possible confounding associated with differences in the number of samples obtained from each farm, factors considered significant on the basis of results of the Fisher exact test were further analyzed by use of the Mantel-Haenszel procedure, with data stratified on the basis of number of samples (ie, greater than or less than median number of samples for all dairies). Again, values of P < 0.05 were considered significant.

Results

Twenty dairy herds were included in the study. Eighteen consisted of Holstein cattle, and 2 consisted of Jersey cattle. Median herd size was 403 cows (range, 22 to 1,310 cows). Median rolling herd average was 16,000 lb (range, 18,000 to 30,400 lb). Median number of samples collected from each farm was 48 (range, 30 to 57). Salmonella organisms were isolated from swab samples from 9 of the 20 (45%) dairies. The proportion of samples from which Salmonella was isolated on the 9 positive dairies ranged from 19% to 98%.

Salmonella serotype Meleagridis was the most commonly identified serotype, being the only isolate on 4 dairies and found in conjunction with S Kentucky on 2 dairies and S Cyprus on 1 dairy. Salmonella serotype Cerro and S Corvallis were each isolated from 1 dairy. All isolates were susceptible to amikacin, amoxicillin-clavulanic acid, ampicillin, ceftiofur, cephalothin, cefazolin, florfenicol, gentamicin, tetracycline, ticarcillin, and trimethoprim-sulfamethoxazole.

Salmonella organisms were recovered from the floors of pens used to house second lactation and older cows (9 dairies), pens used to hold sick cows (7), common alleyways (6), holding areas for the milking parlor (4), and maternity pens (4) and from skid loaders used for manure removal (3).

Production level (greater than or less than the median rolling herd average) was not significantly associated with whether Salmonella organisms would be isolated from the environment (OR, 5.33; 95% CI, 0.67 to 62.14; P = 0.07). Although initial univariate analysis (OR, 9.33; 95% CI, 0.87 to 137.3; P = 0.03) suggested that herd size (greater than or less than median herd size) might be a significant risk factor for isolation of Salmonella organisms, this was demonstrated to be an effect of the larger number of samples obtained on larger dairies. Multivariate analysis incorporating the Mantel-Haenszel procedure to account for the number of samples obtained from each farm indicated that herd size was not significantly associated with whether Salmonella organisms would be isolated (OR, 4.75; 95% CI, 0.55 to 92.2; P = 0.21). Other risk factors that were examined, including manure handling system in the free stall facility (mechanical scraper or slatted floor), whether manure was spread on fields used for forage, whether an animal protein source was incorporated in feed rations for lactating cows, whether milking was performed 2 or 3 times a day, whether common-use equipment was involved in manure handling and feed distribution, and whether the facility was constructed all at once or had been constructed in 2 or more phases. The Fisher exact test was used to test for associations between risk factors and identification of environmental Salmonella contamination; values of P < 0.05 were considered significant. To examine possible confounding associated with differences in the number of samples obtained from each farm, factors considered significant on the basis of results of the Fisher exact test were further analyzed by use of the Mantel-Haenszel procedure, with data stratified on the basis of number of samples (ie, greater than or less than median number of samples for all dairies). Again, values of P < 0.05 were considered significant.
source was incorporated in feed rations for lactating cows, whether milking was performed 2 or 3 times a day, whether common-use equipment was involved in manure handling and feed distribution, and whether the facility was constructed all at once or had been constructed in 2 or more phases, were also not associated with whether *Salmonella* organisms could be recovered from the environment.

**Discussion**

Results of the present study suggest that *Salmonella* spp can be isolated from the environment of free stall dairies in Wisconsin without any history of clinical salmonellosis. In particular, organisms were isolated from 9 of 20 (45%) dairies, with *S* Meleagridis being the most commonly isolated serotype. Our observations were consistent with findings of previous studies demonstrating that animals without clinical signs of infection can shed *Salmonella* organisms and that organisms can be isolated from the environment of animals without clinical signs of disease, sometimes in a protracted and repeatable manner. However, it should also be remembered that serotypes Kentucky, Meleagridis, and Cerro can also be frequently isolated from cattle with clinical signs of salmonellosis and that these 3 serotypes represent the fifth, seventh, and ninth most frequently isolated serotypes from cattle, according to National Veterinary Services Laboratory data, although they represent only approximately 6% of all bovine isolates.12 Environmental contamination and protracted fecal shedding in the absence of clinical disease have also been well documented in association with *S* Typhimurium,2,13 the serotype isolated most commonly from cattle with clinical salmonellosis.12 It is worth noting that we did not isolate any of the more common pathogenic serotypes such as Typhimurium, Newport, or Dublin on any of the farms in the present study. This suggests that environmental contamination with these serotypes is less common in the absence of clinical disease and that the serotypes we did obtain, including Meleagridis, Kentucky, and Cerro, have a weaker association with clinical disease, even in the face of widespread environmental contamination on dairy farms.

Although the physical layout of facilities varied from farm to farm in the present study, there was some repeatability with respect to those environmental sites from which *Salmonella* isolates were obtained. The 2 locations from which organisms were most commonly isolated were pens housing second lactation and older cows (9 dairies) and those used for sick cows (7). High-traffic locations such as alleyways (6) and the holding areas for the milking parlor (4) were also common sites from which organisms were obtained. These observations suggest that older cows and sick cows are more likely to be shedding *Salmonella* organisms into their environment, even though they do not have any signs of enteric disease. Of considerable concern was our observation that *Salmonella* organisms were isolated from the maternity areas of 4 dairies.

When collecting environmental samples from dairy farms, sample handling is important. In the present study, swab samples were placed directly into buffered peptone water, an enrichment medium designed to limit overgrowth of non-*Salmonella* fecal bacteria. This step should be performed prior to incubation and prior to transfer to further selective tetrahydrox or selenite medium.14,15

All *Salmonella* isolates in the present study were susceptible to all antimicrobial agents tested. This was surprising to us, given that in our experience, clinical salmonellosis on Wisconsin dairy farms has been associated with multiple-drug resistant serotypes and multiple-drug resistant *S* Meleagridis and *S* Kentucky strains have been isolated from such farms. Similarly, although these serotypes were not isolated in the present study, multiple-drug resistant strains of *S* Newport and *S* Typhimurium have been identified by other researchers.8,10 This discrepancy between antimicrobial susceptibility patterns for environmental isolates in the present study and isolates obtained from animals with clinical disease is thought-provoking but rather hard to reconcile. It may be that farms experiencing clinical salmonellosis are more likely to have substantial concurrent antimicrobial use, potentially promoting the development of antimicrobial resistance. However, it subjectively appeared that antimicrobial use on the farms in the present study was average for the industry, with agents from the β-lactam, tetracycline, macrolide, and sulfonamide groups being used.

None of the factors examined in the present study was significantly associated with whether *Salmonella* organisms would be isolated from the environment. The small number of herds in the study was perhaps an obstacle to identifying such factors. In a previous study,8 for instance, a larger herd size was associated with a greater likelihood of detecting *Salmonella* spp in the feces of cattle without clinical signs of disease. Further research is indicated to examine risk factors that may be associated with an increased likelihood of environmental *Salmonella* contamination and clinical salmonellosis.

Our observation that certain areas were the most common locations from which *Salmonella* organisms were obtained should not be surprising. A recent study8 of dairies in New York that had recently had outbreaks of clinical salmonellosis identified the pens for holding sick cows and the pens for holding cows that had recently calved as the environmental areas from which *Salmonella* organisms were most frequently isolated. Taken together, these findings emphasize the importance of controlling traffic patterns and cattle movement on large dairies to minimize the exposure of high-risk groups to *Salmonella* organisms and other enteric and respiratory tract pathogens.

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**References**


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