PEER REVIEWED

Salmonella Contamination of Rubber Boots Worn on Dairies

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Abstract

Rubber boots were cultured for salmonella after being worn in calving, hospital and fresh cow pens on 27 selected dairies. Dairies were selected on the basis of willingness to cooperate, previous isolation of salmonella from bulk tank milk, or recent history of clinical salmonellosis. Six salmonella serotypes, including *Salmonella typhimurium* and *Salmonella newport,* were isolated from boots worn on 12 of the dairies. Salmonella was re-isolated from one of four boots 48 hours after they were casually washed. Veterinarians should be aware of the potential to transfer microorganisms from one location to another on boots and inform dairymen as they develop biosecurity plans to minimize this hazard.

Résumé

Des échantillons provenant de bottes de caoutchouc ont été mis en culture pour l'identification de salmonelles après que les bottes eurent été portées dans des enclos de vêlage, d'hôpital et de vaches fraîchement vêlées sur 27 fermes laitières choisies. Les fermes laitières ont été sélectionnées en fonction de l'empressement à collaborer, de l'isolement au préalable de salmonelles dans le lait du réservoir ou de l'identification récente de salmonellose clinique. Six sérotypes de salmonelles, incluant *Salmonella typhimurium* et *Salmonella newport*, ont été isolés de bottes portées dans 12 fermes laitières. Des salmonelles ont été isolées à nouveau d'une botte sur quatre, 48 heures après avoir été lavées normalement. Les vétérinaires devraient être mis au courant du potentiel de transfert des microorganismes d'un lieu à un autre par les bottes de travail et devraient en informer les producteurs laitiers lors de l'implantation de plans de bio-sécurité pour minimiser le risque de transmission.

Introduction

Recently there has been much attention given to biosecurity on dairies, both in lay[3,7,8] and scientific[1,4] literature. The concern has been to prevent introduction of new pathogens such as salmonella, hairy heel warts, *Staphylococcus aureus* or mycoplasma mastitis, Johne's Disease, and particularly during the spring of 2001, Foot and Mouth Disease. While these concerns are valid, there is also an equally important need for within-dairy biosecurity strategies to prevent movement of potential pathogens between cattle within the dairy.

The purpose of a dairy biosecurity plan is to reduce disease in dairy livestock and reduce the number of market dairy cattle arriving at slaughterhouses contaminated with potential food-borne pathogens. The objective of this study was to determine the extent that salmonella organisms contaminate rubber boots worn by dairy workers as they move about in the calving, hospital and fresh cow pens on dairies.

Materials and Methods

Herd selection. Herds were selected for the study based on either the occurrence of salmonella in bulk tank milk or recent evidence of clinical salmonellosis in adult cows. With the assistance of county dairy livestock advisors (Cooperative Extension Service), dairy owners were contacted based on the likelihood that the dairymen would cooperate in the study. Dairymen on 28 dair-

ies were solicited to participate in the study, and 27 allowed us to conduct the study on their dairy.

Sample collection. On each dairy, a new pair of rubber boots taken directly from the shipping box was used. New boots were assumed to be free of salmonella, however, they were not cultured prior to use. In each case, the boots were walked through the calving, hospital or fresh cow pens. When possible, we walked in the flush alley behind the cows as they were locked-up for feeding. Otherwise, we walked through the alleys and pens, attempting to walk through as many manure pats as possible. After approximately 25 paces, sterile cotton swabs were passed across the sides, toes and bottoms of one boot. The walking procedure was repeated and the other boot sampled. Between six and eight swabs were collected from the boots on each dairy. Similar samplings were done on some boots 48 hours after initial sample collection.

Microbiological methods. To isolate salmonella spp. from feces, a sterile cotton-tipped swab was used to collect approximately 0.1–0.2 g of fecal material from each site on the boot. The same methods were used to sample boots both on the dairies and those sampled 48 hours later. Each swab was cultured individually. Samples were enriched in 10ml of Tetrathionate Broth (TTB)[a] at 99°F (37°C) for 20 hours. Subcultures from the TTB were then plated to Brilliant Green[b] and Xylose-Lysine Desoxycholate[b] (XLD) agar plates and incubated overnight at 99°F (37°C). On Brilliant Green (BG) agar, suspect salmonella colonies were non-lactose fermenting, pink-white opaque colonies with a red background. On XLD agar, suspect salmonella were colonies that produced hydrogen sulfide. Two suspect colonies were picked from each plate and placed onto Triple Sugar Iron Agar slants[a] and Urea Agar slants[a] for further classification. Samples that showed typical salmonella reactions for these tests were re-streaked onto Blood Agar[b] to check for purity of the strain. Serologic identification of isolates was done using a slide agglutination test with anti-sera to serogroups B, D, E, C1, C2 and C3. Isolates classified as *Salmonella sp.* were submitted for somatic and flagellar serotyping.

Bulk tank milk from the participating dairies was collected from the two creameries in the tri-county area. Samples less than 48 hours old were picked up from each creamery laboratory. Each sample was shaken vigorously by hand, then 1 ml was pipetted into 9 ml of Tetrathionate Broth. Samples were incubated at 99°F (37°C) for approximately 20 hours. Following incubation each sample was streaked on Xylose-Lysine Desoxycholate agar plates and Brilliant Green agar plates and then incubated at 99°F (37°C) overnight. Suspect colonies from the XLD and BG plates were restreaked on Blood Agar and biochemically tested using Triple Sugar Iron Agar slants and Urea Agar slants. All slants and plates were incubated at 99°F (37°C) overnight. Colonies yielding the appropriate biochemical reactions for salmonella were serogrouped using Somatic O antigen for groups A, B, C, D E_1, E_2, E_3, E_4, L, C_1, C_2 and C_3. Isolates where then submitted for further somatic and flagellar serotyping.

[a]Difco, Sparks, Maryland
[b]Remel Inc, Lenexa, Kansas

Results

No salmonella were isolated from boots on 15 of the 27 dairies. Five different salmonella serotypes were isolated from boot swabs collected on the remaining 12 dairies (Table 1). On seven dairies, two different salmonella serotypes were isolated, and on five dairies only a single serotype was found. Boots from four dairies were re-cultured 48 hours after being hosed-off in the dairy parlor using drop-hoses and repacked into the shipping boxes. Salmonella was cultured from one of the four repacked boots.

On the nine dairies with previous bulk tank milk isolates of Salmonella, the same Salmonella serotypes were isolated from the boots and the bulk tank milk (Table 2). Additional salmonella serotypes were found on boots on six of the nine dairies that were different from those found in the bulk tank milk. On two additional dairies, boot sampling was conducted as they were experiencing clinical salmonellosis in adult cattle. *S. newport* was found in both the cattle and on the boots. Samples from boots were also taken from a dairy adjacent to the one experiencing clinical salmonellosis, however, two different serotypes of salmonella were found on that dairy (*S. montevideo* and *S. meleagridis*).

Discussion

Several steps were taken to increase the likelihood of finding salmonella on the boots during this study. First, most of the study dairies were selected because

Table 1. Distribution of salmonella serotypes isolated from boot swabs taken on 27 California dairies during December 2000 through April 2001.

Salmonella serotypes	Number of isolates
S. montevideo	10
S. meleagridis	4
S. newport	2
S. typhimurium	1
Untyped	1

Table 2. Salmonella isolates found in bulk tank milk and on boots.

Number of dairies	Bulk tank milk	Boots
5	*S. montevideo*	*S. montevideo*
2	*S. montevideo*	*S. montevideo, S. meleagridis*
1	*S. montevideo, S. meleagridis, S. typhimurium*	*S. montevideo,* untyped
1	*S. meleagridis*	*S. meleagridis, S. montevideo*

they were known to have salmonella in the bulk tank milk. Other dairies had recently experienced clinical cases of salmonellosis. This selection method increased the likelihood of finding salmonella as compared to random sampling dairies with unknown salmonella background. Secondly, we walked the boots through pens occupied by cows believed to be at high risk for shedding salmonella; specifically in pens that housed cows under stress due to parturition and disease.[6]

On 92% of the dairies where salmonella was cultured from boots, the same salmonella serotypes were found in bulk tank milk and clinically affected cows. This suggests that the salmonella we detected in the environment were biologically related to the salmonella associated with the cows, and not just environmentally adapted serotypes.

While it is known that salmonella-positive cows will contaminate the dairy environment, the findings from this study reinforce the importance of developing within-dairy biosecurity programs to control movement of salmonella between management areas on the dairy. It would be useful to know the amount of salmonella carried on boots to other locations, however, boots only serve to transfer the salmonella. On most dairies, the salmonella transferred by contaminated boots can grow and multiply to an amount sufficient to cause infection. Within-dairy biosecurity efforts will augment previously suggested strategies of control focused on rodent and bird control, commodity feeds and carrier animals.[5]

Results of this study provide science-based evidence that veterinarians can use to persuade dairy clients to consider within-dairy biosecurity plans. Possible strategies are 1) to wear disposable boots when working with either susceptible or infected high-risk animals, and discarding them before leaving the area; 2) to wear rubber boots and leave them in the high-risk area before moving to another area; or 3) thoroughly removing all manure from the boots and disinfecting them with an appropriate disinfectant before moving to another area. Another alternative for larger dairies is to restrict movement of workers from high-risk to low-risk areas. Different methods may be applicable for visitors and farm workers. For instance, disposable boots may be suitable for short farm tours by visitors, but may not be sturdy enough for routine, sustained use by dairy employees. On a dairy-by-dairy basis, dairy producers and their veterinarian should do a risk analysis, as well as a cost analysis, to determine what method of within-dairy biosecurity is appropriate.

Finding salmonella contamination on boots 48 hours after the initial isolation indicates that salmonella can survive on boots, presumably in small deposits of manure. This strongly suggests that merely washing boots with drop-hoses in the dairy parlor is an inadequate method for removing salmonella from boots. Thorough cleaning of boots may take 5 to 10 minutes of scrubbing to remove all manure, with particular attention given to the rough boot bottoms. Scrubbing should be followed by chemical disinfection to further reduce the risk of spreading salmonella. Disinfection is also time consuming and may require up to five minutes of contact time to be effective.[2] Outlining strategies for cleaning and disinfection may provide an opportunity for the veterinarian to be involved in on-farm biosecurity that may reduce the risk of spreading salmonella on dairies they serve.

Conclusions

Beyond the need for biosecurity, the message for veterinarians, as well as others such as AI technicians or others that may visit multiple dairies, is straightforward. Boots should be thoroughly washed to remove all manure and properly disinfected prior to arrival at a dairy. After working on the dairy, the boots should be thoroughly washed to remove all manure and then disinfected before moving to another location on the dairy or leaving the dairy. An alternative strategy is to have the dairy boots that remain on the dairy.

Acknowledgement

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Abstracts

Clinical Features of Renal Tubular Dysplasia, a New Hereditary Disease in Japanese Black Cattle

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A new hereditary disease characterized by renal failure, poor growth and long hooves in Japanese Black cattle (wagyu) has been recognized in a region of central Japan since 1990. The number of calves affected has increased gradually, with the incidence reaching 17 of 485 (3.51 percent) in 1995. Almost all the calves were slightly undersized at birth, and repeatedly had diarrhoea during the neonatal period. They began to show signs of growth retardation with proportional body and elongation of the hooves from about two to five months of age, but they had an almost normal or only slightly decreased appetite. The concentrations of urea nitrogen, creatinine and inorganic phosphorus in serum were high, and the affected calves excreted diluted urine frequently. Among 25 cases, the urine of 21 contained occult blood, 24 contained protein and two contained glucose. In 29 calves observed for 30 to 130 days, the course of the disease varied; in 21 of them it remained unchanged, six became gradually worse and two became severely debilitated and died. The disease was diagnosed as renal tubular dysplasia by histopathological examination.

Aetiology of Reduced Milk Ejection in Cows After Transport and the Use of a Long-Acting Analogue of Oxytocin for Prophylaxis

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Milk flow was recorded in 21 cows for three days after they were admitted to a large animal hospital. When the spontaneous flow of milk had stopped, a physiological dose (1 iu) of oxytocin was administered intravenously. Five of the cows were, in addition, treated with 0.35 mg of long-acting analogue of oxytocin (carbetocin) one hour before the first milking after they were admitted. In the 16 cows not treated with carbetocin, only about 30 percent of the total milk yield was released spontaneously on the first day, and the injection of 1 iu of oxytocin released approximately another 60 percent of the total milk yield. On the second day, the proportion of the total milk yield released spontaneously increased and the fraction released after the injection of 1 iu oxytocin decreased. In contrast, the five cows treated with carbetocin released on average 94 percent of the total milk yield spontaneously during the first milk.