Sources of Intramammary Infections from *Staphylococcus aureus* in Dairy Heifers at First Parturition¹

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ABSTRACT

The study objective was to identify probable sources and modes of transmission of 91 Staphylococcus aureus isolates obtained from the colostrum of 76 heifers at parturition. Sources cultured were milk (including colostrum), heifer body sites (teats, muzzle, rectum, vagina, and lacteal secretions), and environmental sites (bedding, insects, housing, water, feedstuffs, humans, nonbovine animals, air, and equipment). Staphylococcus aureus isolates were characterized by 63 phenotypic traits. A similarity coefficient was calculated by herd to identify the S. aureus that most closely resembled the S. aureus obtained from heifer colostrum. Staphylococcus aureus from a heifer's colostrum was compared with all preexisting S. aureus isolates from that heifer's herd. Isolates that were ≥90% similar were considered to be identical. Because 30 (of the 91) S. aureus isolates from heifer colostrum were collected prior to environmental sampling, only 61 S. aureus isolates from heifer colostrum were available for comparison among all three sources. Possible sources of S. aureus from heifer colostrum at parturition were milk (70%, 43 of 61 isolates), heifer body sites (39%, 24 of 61), environmental sites (28%, 17 of 61), or no identified source (16%, 10 of 61). Three heifers with intramammary infection (IMI) from S. aureus at parturition had the same S. aureus on their teats prior to parturition. Milk was the only source identified for 41% (25 of 61) of isolates from heifer colostrum. Isolates from heifer body sites were the only source identified for 5% (3 of 61) of heifer colostrum isolates. Staphylococ*cus aureus* from the environment was never the sole possible source for *S. aureus* from heifer colostrum. Data suggest that the major sources of *S. aureus* IMI in heifers at parturition are milk and heifer body sites. Contact among heifers may be an important mode of transmission of *S. aureus* leading to IMI in heifers at parturition.

(**Key words**: heifer mastitis, *Staphylococcus aureus*, mastitis sources, transmission)

Abbreviation key: **HPSA** = herds with a high prevalence of *Staphylococcus aureus*, **LPSA** = herds with a low prevalence of *Staphylococcus aureus*, **RTD** = routine test dilution.

INTRODUCTION

Staphylococcus aureus is the most prevalent major mastitis pathogen in the US and several other countries (3). Although a great deal of progress in mastitis control has taken place over the years, IMI from *S. aureus* still occur in >80% of dairies (8, 23), even though *S. aureus* IMI has been reduced to <5% of cows in many herds (7, 10, 18, 22). Some herds claim complete eradication, yet others that practice all the standard techniques for hygiene at milking and have mean SCC of <200,000 cells/ml have been unable to eradicate this organism (11).

One reason for the failure to eradicate *S. aureus* IMI may be that the control measures for mastitis do little to control this disease in prepartum heifers. Thus, heifers with *S. aureus* IMI at parturition may represent a deterrent to eradication of *S. aureus*. Not only do they add to the prevalence of *S. aureus* IMI in the lactating herd, but those heifers may also act as reservoirs of this contagious pathogen, which can be transmitted to uninfected herdmates (16). Heifers with *S. aureus* IMI at parturition represent nearly one-third of the new cases of *S. aureus* IMI in dairy herds (16).

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Site of	Herd							
origin	A1	B1	С	D	Е	F	\mathbf{G}^1	
	(no. of isolates)							
Milk ²	12	75	69	100	157	52	20	
Colostrum ³	5	27	30	10	18	1	2	
Udder skin ⁴	24	19	28	330	21	7	36	
Muzzle ⁴	5	27	4	87	11	31	28	
Rectum ⁴	2	0	0	8	1	0	4	
Vagina ⁴	1	1	3	19	5	4	2	
Lacteal secretion ⁴	0	5	3	4	0	0	0	
Bedding ⁵	0	0	2	1	1	2	0	
Insects ⁵	0	0	2	3	8	2	0	
Housing ⁵	1	0	2	1	0	3	0	
Water ⁵	0	0	0	1	1	0	0	
Feedstuffs ⁵	0	1	3	4	0	1	0	
Man ⁵	4	1	0	0	0	2	5	
Animals other than cattle ⁵	0	0	3	0	0	2	1	
Air ⁵	2	0	1	0	0	0	1	
Equipment ⁵	1	1	2	2	2	0	0	

TABLE 1. Origin of Staphylococcus aureus isolated from seven northwestern dairies.

¹Herds with low prevalence (\leq 5%) of IMI from *S. aureus*; others have high prevalence (>10%). ²From milk and colostrum collected from lactating cows and heifers (after first milking).

³From colostrum collected from heifers immediately prior to first milking.

 $^{4}\mathrm{From}$ body sites of prepartum heifers collected over 18 mo period from May 1989 to November 1990.

⁵From environmental sites collected over 11 mo from January 1990 to November 1990.

Control measures such as prepartum teat dipping, prevention of suckling, and feeding milk that is free of *S. aureus* have not been successful in controlling this disease (1, 5, 19, 20), and the development of successful control measures requires an understanding of the epidemiology of *S. aureus* IMI in heifers.

The purpose of this study was to identify the most likely sources and modes of transmission of *S. aureus* for heifers that had *S. aureus* IMI at parturition.

MATERIALS AND METHODS

Herd Selection

Seven herds were selected based on a past history of *S. aureus* IMI and a willingness to cooperate in the project. Six herds milked only Holstein cows, and one herd milked Holstein and Brown Swiss cows. Herd size ranged from 80 to 375 milking cows. Based on the initial herd results of milk culture (16), low prevalence herds (**LPSA**) were defined as having had a prevalence of *S. aureus* IMI of \leq 5%. High prevalence herds (**HPSA**) were defined as having had a prevalence of *S. aureus* IMI >10%. Three herds were classified as LPSA herds, and four herds were classified as HPSA herds.

Staphylococcus aureus isolates. Collection of samples, isolation, and identification of *S. aureus*

have been previously described (16, 17). Origins of S. aureus by herd are presented in Table 1. Staphylococcus aureus from heifer colostrum indicated that S. aureus was isolated between first parturition and first milking. Three major sites were cultured for S. aureus: milk (included colostrum) of lactating cows or lactating heifers, heifer body sites, and environmental sites. Staphylococcus aureus isolates from heifer colostrum were compared only with S. aureus collected before the parturition date for each individual heifer. Milk samples were collected at parturition, just prior to the nonlactating period, during whole herd culturing, and during clinical mastitis. The teat skin, lacteal secretions, the muzzle area, the vagina, and the rectum were the heifer body sites sampled. Bedding, insects, housing, water, feedstuffs, humans, animals other than cattle, air, and equipment were the environmental sites sampled. Time periods for sample collection have been detailed previously (17). Briefly, milk samples were collected from June 1988 to July 1992 (4 yr); sampling of heifer body sites occurred once during summer 1989 and then once during each season thereafter, beginning with the first 2 mo of 1990 (winter sampling period); environmental sampling began with the winter sampling period and continued at the same time as heifer body site sampling thereafter. Staphylococcus aureus were stored frozen $(-20^{\circ}C)$ in nutrient broth pending further analysis. *Staphylococcus aureus* were characterized by biotyping, antibiograms, and phage typing.

Phenotypic Characterization

Immediately prior to characterization, *S. aureus* were thawed at room temperature (ca. 28°C) and isolated in pure culture on Columbia blood agar containing 5% sheep blood (BBL, Becton Dickinson, Cockeysville, MD). *Staphylococcus aureus* from a single herd were analyzed and characterized as a group to help avoid day-to-day test variation.

Biotyping. The API STAPH Trac system (Analytab Products, Plainview, NY), which includes 19 biochemical tests for the speciation of *Staphylococcus* from human and veterinary origin, was used. The instructions of the manufacturer were followed. Usually the results for each of the 19 tests were clearly negative or clearly positive; however, when a test was equivocal, it was considered a weak positive. For the purposes of characterization, reactions were recorded as negative = 0, weak positive = 1, and positive = 2 for each of the 19 biochemical tests.

Antibiogram. Antibiotic sensitivity patterns were determined by the disk diffusion method previously described (2). The 12 antimicrobial discs (Difco, Detroit, MI) used were ampicillin, cephalothin, clox-acillin, erythromycin, gentamicin, novobiocin, nitrofurantoin, penicillin, streptomycin, tetracycline, lincomycin, and neomycin. Results were measured and recorded as the diameter of the zone of inhibited growth of *S. aureus* around the antimicrobial discs in millimeters. This procedure has been determined to be reliable and repeatable (9).

Phage typing. Phage typing was performed using the 16 phages of the international bovine set (6) as previously described (15). Both routine test dilution (**RTD**) and 100 times RTD were performed. Results were recorded as \geq 50 plaques = 2, \geq 10 and <50 plaques = 1, and <10 = 0 plaques. Thus, for each *S. aureus*, a 32-character phage pattern of 0, 1, or 2 at RTD and 100 times RTD was determined.

Data Analyses

A similarity coefficient was calculated by herd between each *S. aureus* isolates from heifer colostrum and all other preexisting *S. aureus* isolates from that herd. Because the antibiogram inhibition zone was measured in millimeters, some variation was expected (9). *Staphylococcus aureus* isolates differing by >5 mm were considered to be different, and *S. aureus* isolates differing by \leq 5 mm were considered to be identical for that specific antibiotic. The 5-mm variation was based on the mean of 2 standard deviations (seven replicates) of each antibiotic tested of S. *aureus* strain 27543 from the American Type Culture Collection (Cockeysville, MD). For biochemical and phage characters, S. aureus that differed for a particular character were considered dissimilar for that character, and *S. aureus* that were identical for a particular character were considered identical for that character. Thus, two S. aureus that were identical on biochemical and phage characters and that had ≤ 5 mm difference in the inhibition zone of any of the 12 antibiotics would be 100% identical. Only S. aureus isolates known to exist prior to parturition were considered as possible sources of S. aureus IMI of that heifer at parturition. The chi-square test (Statistix 3.1; Analytical Software, St. Paul, MN) was used to test differences in major sources between herd groups. Major sources were milk, heifer body sites, and environmental sites.

Interpretation

The purpose of the similarity coefficient is to establish the most likely sources of *S. aureus* that infect the mammary glands of heifers prior to first parturition. *Staphylococcus aureus* collected prior to *S. aureus* from heifer colostrum with a similarity of \geq 90% were considered as possible sources or as having possible roles in the transmission of *S. aureus* to prepartum heifers. For the purpose of analysis, *S. aureus* with \geq 90% similarity were considered the same.

RESULTS

Ninety-three unique *S. aureus* isolates were cultured from colostrum of heifers at parturition. Two of these were resistant to lysis by all phages (nontypeable) and were omitted from further study.

The potential sources of 61 of the S. aureus isolated from heifer colostrum after the first environmental sampling period are presented by herd group in Table 2. The HPSA herds had a significantly higher percentage of isolation from environmental sources than did the LPSA herds (P < 0.05). The percentage of S. aureus from other sources did not differ significantly between herd groups. Of 61 S. aureus from heifer colostrum, 70% (43 of 61) were the same as preexisting S. aureus from milk, 39% (24 of 61) were the same as preexisting *S. aureus* from heifer body sites, 28% (17 of 61) were the same as preexisting and S. aureus from environmental sites; for 16% (10 of 61), the source was unknown. The sum of the numerators was >61 because S. aureus from heifer colostrum could be $\geq 90\%$ similar to more than one source.

TABLE 2. Possible sources of Staphylococcus aureus IMI at parturition in heifers.

Source	HPSA ¹	LPSA ²	All herds
	(%) (no./no.) ³	(%) (no./no.) ³	(%) (no./no.) ³
Milk ⁴	75 ^a 30/40	62 ^a 13/21	70 43/61
Heifer body site ⁴	48a 19/40	24 ^a 5/21	39 24/61
Environment ⁴	38a 15/40	10 ^b 2/21	28 17/61
Unknown ^{4,5}	13 ^a 5/40	24a 5/21	16 10/61

^{a,b}Means within rows without common superscript letters differ (P < 0.05).

¹Herds with a lactating herd IMI prevalence of *S. aureus* >10%.

²Herds with a lactating herd IMI prevalence of *S. aureus* \leq 5%.

³Number of isolates from heifer colostrum compared to preexisting *S. aureus* isolates.

 4 Milk or colostrum from both heifers and cows. Total does not include the 30 *S. aureus* from heifer colostrum collected prior to the first environmental sampling period (January 1990).

⁵No preexisting *S. aureus* strains were \geq 90% similar to primiparous cow IMI *S. aureus* strains.

In 41% (25 of 61) of the isolates from heifer colostrum, the only known possible source was *S. aureus* from milk. In 5% (3 of 61) of the isolates from heifer colostrum, the only known possible source was *S. aureus* from heifer body sites. Environmental sources were never the only known source of *S. aureus* from heifer colostrum (0 of 61).

Thirty isolates from heifer colostrum were collected prior to the first environmental sampling, and 61 were collected after the first environmental sampling period. Thus, 91 *S. aureus* isolates from heifer colostrum were compared with preexisting *S. aureus* from milk. Of the 91 *S. aureus* isolates from heifer colostrum, 68% (62 of 91) were the same as preexisting *S. aureus* from milk. Potential sources for 23% (21 of 91) of heifer colostrum isolates were not identified. Twenty-two *S. aureus* from heifer colostrum were collected prior to and 69 after the first heifer body site sampling period. Thus, 69 were compared with preexisting *S. aureus* cultured from heifer body sites. Of the 69 *S. aureus* from heifer colostrum, 38% were the same as *S. aureus* cultured from heifer body sites.

Possible sources of *S. aureus* from heifer colostrum by herd are presented in Table 3. For herds F and G, the phage-typeable *S. aureus* from heifer colostrum were collected prior to body site and environmental sampling periods. In the other five herds, *S. aureus* from milk were common sources for four herds and *S. aureus* cultured from heifer body sites were common sources for 3 herds. Environmental *S. aureus* were potential sources for four of five herds. For six of the seven herds, *S. aureus* sources for some isolates of *S.*

Herd		Source by herd								
	Milk	Milk ¹		Body site ²		Environment ³		Unknown ⁴		
	(%)	(no./no.) ⁵	(%)	(no./no.) ⁵	(%)	(no./no.) ⁵	(%)	(no./no.) ⁵		
A ⁶	0	0/5	67	2/3	0	0/3	60	3/5		
B ⁶	70	19/27	14	3/22	11	2/18	26	7/27		
С	77	23/30	28	7/25	25	6/24	13	4/30		
D	56	5/9	83	5/6	33	1/3	22	2/9		
Е	78	14/18	69	9/13	62	8/13	22	4/18		
F	100	1/1					0	0/1		
G ⁶	0	0/1					100	1/1		

TABLE 3. Possible sources of Staphylcocccus aureus IMI at parturition in heifers by herd.

 $\label{eq:stars} {}^{1} Isolates from heifer colostrum \geq 90\% similar to preexisting isolates from milk and colostrum. \\ {}^{2} Isolates from heifer colostrum \geq 90\% similar to preexisting isolates from heifer body sites.$

³Isolates from heifer colostrum ≥90% similar to preexisting environmental isolate.

⁴Isolates that were not \geq 90% similar with any preexisting *S. aureus* from any source.

⁵Number of isolates from heifer colostrum compared with preexisting *S. aureus* isolates. Milk sources preexisted for all 91 *S. aureus* from heifer colostrum. Body site sources preexisted for 69 *S. aureus*, and environmental sources preexisted for 61 *S. aureus*.

⁶Herds with low prevalence (\leq 5%) of IMI from *S. aureus*; others have high prevalence (>10%).

TABLE 4. Transmission pattern of *Staphylcoccus aureus* to three heifers that had body sites sampled and S. *aureus* IMI at parturition.

	Heifer				
	88	1032	1266		
First identified on dairy Source ¹ Date Similarity, ² %	Milk 8/17/88 92	Teat skin 8/23/90 92–97 ³	Lacteal secretion 1/24/90 97		
First identified on heifer Site ⁴ Date Similarity, %	Teat skin 8/16/90 90–95	Teat skin 8/23/90 92–94	Lacteal secretion 1/24/90 97		
First identified on herdmate Site Date Similarity, %	Teat skin 8/16/90 92–94	Teat skin 8/23/90 92–97	Teat skin 8/21/90 90–92		
Heifer parturition Date	4/2/91	8/27/90	12/31/90		

¹The first identified *S. aureus* that was the same as the *S. aureus* from heifer colostrum.

²Percentage similarity with the *S. aureus* from heifer colostrum. ³The similarity range is due to multiple isolates of *S. aureus* from sites cultures.

 $^{4}\mbox{Heifer}$ body sites included teat skin, lacteal secretion, muzzle area, vagina, and rectum.

aureus from heifer colostrum remain unknown. Among the herds, the percentage of sources for *S. aureus* from heifer colostrum ranged from 0 to 100% for milk sources, 14 to 83% for heifer body site sources, 0 to 62% for environmental sources, and 0 to 100% for unknown sources. The extreme ranges may be a reflection of the small number of *S. aureus* isolates from heifer colostrum in herds A, F, and G.

Of the 76 heifers with S. aureus IMI at parturition, 8 had body sites sampled prepartum. Staphylococcus aureus was recovered from the teat skin of 5 of the 8 prior to parturition. Staphylococcus aureus from heifer colostrum was the same strain as that recovered from the teat skin in 3 of the 5 heifers. The transmission patterns of S. aureus to these 3 heifers (from different herds) are presented in Table 4. In 1 of the 3 (heifer 88), the strain was first identified as a preexisting milk isolate and was later isolated from the teat skin of heifer 88 as well as from 5 other heifers of similar age. Heifer 88 had an IMI with this strain at parturition 8 mo later. Strains were first identified from the teat skin and a lacteal secretion of heifers 1032 and 1266, respectively. No preexisting milk isolates were identified for these 2 heifers (heifers 1032 and 1266). Heifer 1266 appears to have been colonized nearly 1 yr prior to her S. aureus IMI at parturition. The S. aureus strains cultured from these 3 heifers were also cultured concurrently or subsequently from the teat skin of other heifers of similar ages.

DISCUSSION

Sources

The goal of this study was to identify the most likely sources of *S. aureus* IMI in heifers at parturition. The most common potential sources identified for *S. aureus* mastitis in heifers at parturition were *S. aureus* from milk. This result is consistent with historical assumptions that the mammary gland infected with *S. aureus* is the source of *S. aureus* IMI in heifers at parturition (14, 19).

Staphylococcus aureus on heifer body sites may be an important source of IMI of heifers. Other studies have clearly demonstrated that *S. aureus* is present on body sites of prepartum heifers from birth to first parturition (4, 13, 17, 21). Additional evidence from the current study for heifer body site sources are supported by the 5% of *S. aureus* from heifer colostrum for which the only known source was heifer body sites.

Although *S. aureus* from heifer colostrum were the same as 28% (17 of 61) of environmental *S. aureus*, only 7 of the 17 were found during the same sampling period as *S. aureus* from the environment. *Staphylococcus aureus* from the environment were never the only possible source for *S. aureus* from heifer colostrum. Thus, environmental sources of *S. aureus* are possible but appear to be less likely sources of *S. aureus* from heifer colostrum.

Overall, 24% of S. aureus from heifer colostrum appear to be novel strains. Although it is not surprising that sources could not be identified, some (n = 7)S. aureus from heifer colostrum collected in 1992 (sample collection terminated in July 1992) were not the same as any preexisting S. aureus. This result either suggests that entirely new strains are introduced by heifers at parturition or suggests the limitations of the sampling technique in isolating all strains of S. aureus on a dairy. The strains from each dairy should have included essentially all existing IMI strains. However, only a proportion of heifers in each location had body sites sampled, and body sites of lactating cows were not sampled. Thus, it is entirely possible that distinctly different strains were present on body sites of unsampled cattle.

Mode of Transmission

The consumption of milk containing *S. aureus* by preweaned heifer calves is one obvious route of *S. aureus* transmission. However, results from a previ-

ous study do not support this premise because no preweaned heifers with *S. aureus* isolated from a body site were persistently colonized from birth to first parturition (17). Although S. aureus from milk may be an important source of S. aureus from heifer colostrum, little evidence was gained as to how this transmission occurs (in 41% of 61 heifers, only S. aureus from milk were the same). One plausible explanation is social contact among prepartum heifers. Evidence to support this explanation is presented in Table 4, in which the strain identified in heifer colostrum at parturition was also identified on body sites of multiple heifers of similar age. Prepartum heifers in herd G were partially raised in a heifer rearing feedlot, and social contact with heifers from numerous other dairies may explain the presence of newly acquired strains in this low prevalence dairy herd.

Role of Environment

Evidence for the role of environmental *S. aureus* is less substantial but merits discussion. Two heifers from herd B with *S. aureus* IMI at parturition had the same strain as that isolated from the nose of a dairy worker, suggesting that humans may play a role in the transmission of *S. aureus* to cattle. Humans may be persistently colonized with *S. aureus* in the anterior nares (12).

Staphylococcus aureus was cultured from 8 flies captured at dairy E. Staphylococcus aureus from flies were >90% similar to 8 of the S. aureus from heifer colostrum. However, these fly isolates were collected 1 yr prior to S. aureus from heifer colostrum. During the sampling period (summer 1990) when S. aureus from flies were being collected, the heifers that later had S. aureus IMI at parturition were on a farm several miles away. Thus, these flies were probably not responsible for the transmission of S. aureus to these heifers. However, S. aureus from flies were also the same as S. aureus from milk and from body sites of preweaned heifer that were maintained on the same farm. Although the evidence is not clear, the data suggest that the flies may be important vehicles of transmission. Only in herd E were the S. aureus from flies the same as S. aureus from heifer colostrum.

Herd G had only 1 primiparous cow with a typeable *S. aureus* IMI strain at parturition. This strain became a predominant strain on this dairy and was primarily isolated from body sites of heifers. The strain was also recovered from the air around the group of breeding age heifers. Identification of the same strain of *S. aureus* from the air as that isolated

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from prepartum heifer teat skin suggests a possible means by which *S. aureus* may be transmitted among prepartum heifers. The muzzle of prepartum heifers was a common site of *S. aureus* colonization identified in a previous study (17). Although *S. aureus* of the same strain as found in heifer colostrum was recovered from bedding, housing, and feedstuffs, the epidemiologic role of those items is not clear. *Staphylococcus aureus* recovered from water, from animals other than cattle, and from equipment were not of the same strain as identified in heifer IMI at parturition.

CONCLUSIONS

This study is limited to suggesting the most plausible sources of *S. aureus* that cause IMI in heifers at parturition and attempting to explain the mode of transmission. Milk from the infected mammary gland and heifer body sites appear to be the major sources of *S. aureus* for IMI in heifers. The data also suggest that the persistent colonization of the teat skin occurs and may be an important predisposing factor for *S. aureus* IMI at parturition.

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