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Evaluation of the Risk of Transmitting *Staphylococcus aureus* Strains between Replacement Heifers through Commingling at a Heifer-rearing Facility

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Abstract

The objective of this study was to evaluate transmission of *Staphylococcus aureus* (*S. aureus*) strains between heifers that originated from either a high- or low-prevalence *S. aureus* herd after commingling at a heifer-rearing facility. Three to four-month-old heifers from the University of Pennsylvania (UPa) and the University of Delaware (UDel) were transported on a bimonthly basis and raised commingled at Rutgers University. Preliminary work established unique baseline strains of *S. aureus* at both universities prior to commingling, and a high prevalence of *S. aureus* at the UPa (22.4%) and a low prevalence of *S. aureus* at the UDel (2.7%). Composite milk samples were obtained for culture of *S. aureus* from both universities three times in 2006 and four times in 2007. Strain typing of *S. aureus* was performed by pulsed-field gel electrophoresis. The predominant strain isolated at the UPa was not spread to the UDel when heifers were commingled. The prevalence of *S. aureus* at the first routine culture after parturition (3-124 days after parturition) in primiparous cows previously commingled was similar in both herds. Sixty percent of the *S. aureus* strains isolated from commingled primiparous cow from the UPa were strains that were also found in the lactating herd. The UPa had significantly more *S. aureus* infections acquired in later lactation or subsequent lactations in primiparous cows that had been previously commingled and negative at the first routine culture after parturition, and from cows previously not commingled, than the UDel. The predominant strain of *S. aureus* isolated in these cows was the same as the predominant strain isolated from the preliminary baseline cultures. In this study, three to four-month-old commingled heifers returned to their herd of origin two to seven months prior to parturition were more at risk of acquiring an endemic strain of *S. aureus* than strains they may have potentially been exposed to at a commercial heifer-rearing facility. In this study, low-prevalent strains not found in the lactating herd and isolated from primiparous cows at parturition were of minor importance, and the infected mammary gland of lactating cows was the major reservoir of *S. aureus*.

Keywords: bovine, *Staphylococcus aureus* strains, heifers, primiparous cows

Résumé

L'objectif de cette étude était d'évaluer la transmission de souches de *Staphylococcus aureus* (*S. aureus*) entre taures provenant de troupeaux à prévalence élevée ou basse de *S. aureus* suite à leur agrégation dans un élevage de taures. Des taures de trois à quatre mois de l'université de Pennsylvanie (UP) et de l'université du Delaware (UD) étaient transportées à tous les deux mois et élevées ensemble à l'université Rutgers. Des travaux préliminaires avaient établi que les souches de *S. aureus* étaient uniques à chaque établissement avant l'agrégation et que la prévalence de *S. aureus* était plus élevée à l'UP (22.4%) qu'à l'UD (2.7%). Des échantillons composites de lait ont été obtenus pour la culture de *S. aureus* de chacune des universités à trois reprises en 2006 et à quatre reprises en 2007. La détermination des souches de *S. aureus* a été faite avec l'électrophorèse sur gel en champs pulsé. La souche dominante isolée de l'UP ne s'est pas propagée aux taures de l'UD après l'agrégation. La prévalence de *S. aureus* à la première culture de routine suivant la parturition (de 3 à 124 jours après la parturition) chez les taures primipares qui avaient été agrégées était similaire dans les deux troupeaux. Un total de 60% des souches de *S. aureus* isolées des taures primipares agrégées de l'UP étaient

des souches que l'on retrouvait aussi dans le troupeau en lactation. La prévalence d'infection à *S. aureus* acquise plus tard dans la lactation ou dans les lactations subséquentes était plus élevée chez les taures primipares de l'UP, négatives au premier test de routine après la parturition et qui avaient été agrégées ou qui n'avaient pas été agrégées du tout, que chez les taures de l'UD. La souche prédominante de *S. aureus* isolée chez ces taures était la même que celle isolée lors de l'étape préliminaire. Dans cette étude, les taures de trois à quatre mois qui ont été agrégées et ensuite retournées à leur troupeau d'origine deux à sept mois avant la parturition étaient plus à risque d'acquérir des souches endémiques de *S. aureus* que des souches auxquelles elles auraient pu être exposées durant l'agrégation dans un élevage commercial. Dans cette étude, des souches peu prévalentes qui n'étaient pas présentes dans le troupeau en lactation et isolées des taures primipares à la parturition avaient peu d'importance et la glande mammaire des vaches en lactation représentait le plus grand réservoir de *S. aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) is a frequently isolated pathogen from bovine intramammary infections,[23] and mastitis is the most economically important disease in the dairy industry.[2] The calculated loss due to decreased milk production in cows with subclinical mastitis caused by *S. aureus* has been estimated to be $161/cow annually.[2] *S. aureus* is a common contagious organism that causes mastitis in dairy cattle, and 90% of herds have at least one infected cow.[6] *S. aureus* accounted for approximately 10% of isolated pathogens in a New York/Pennsylvania study from 1991-1995.[23] In the 2007 US Department of Agriculture National Animal Health Monitoring Systems study, *S. aureus* was the most prevalent contagious organism isolated, and was found in 43% of dairies studied.[10]

The primary reservoir of *S. aureus* is generally accepted to be the infected cow's mammary gland. Despite adoption of proven control procedures aimed at eliminating the spread of the bacteria between cows at milking time, mastitis caused by *S. aureus* continues to be a major economic problem for many dairy producers[2,23] and a major biosecurity risk in expanding herds through the purchase of infected cows.[24]

Different strains of *S. aureus* can be identified using pulsed-field gel electrophoresis (PFGE), and many different strains of *S. aureus* have been isolated from cows with mastitis.[8] However, several studies have suggested that only a few closely related strains are responsible for most cases of *S. aureus* mastitis over a broad geographic distribution.[3,5,11] There is evidence that some highly transmissible *S. aureus* strains may be more difficult to eradicate,[1] and these strains likely have characteristics that enable them to overcome defenses in milk and establish infection in the mammary gland. Aarestrup *et al*[1] showed that more prevalent strains of *S. aureus* had superior abilities to resist phagocytosis by bovine neutrophils and hence evade host defense mechanisms.

A number of reports have suggested that replacement heifers may serve as a major reservoir of *S. aureus* on dairy farms,[16,22] but the epidemiology of the infections in the replacement heifers is not well understood. The prevalence of *S. aureus* intramammary infections (IMI) in primiparous cows at parturition has been shown to range from 2 to 37%.[14,15,16,22] Roberson *et al*[16] reported approximately one-third of new cases of IMI caused by coagulase-positive *Staphylococcus* were due to primiparous cows at parturition. *S. aureus* was found to be ubiquitous in the environment, and herds with a high prevalence of IMI caused by coagulase-positive *Staphylococcus* had a higher prevalence of *S. aureus* in the environment compared to herds with a low prevalence of IMI caused by coagulase-positive *Staphylococcus*.[17] *S. aureus* has been shown to colonize body sites of heifers at all ages in both low- and high-prevalence herds, and heifers with teat skin colonized by *S. aureus* have a 3.34 times greater risk of IMI caused by *S. aureus* at parturition than do heifers without teat skin colonization of *S. aureus*.[17]

In spite of reports that suggest replacement heifers are a major reservoir of *S. aureus* on dairy farms,[16,22] the epidemiology of *S. aureus* IMI in replacement heifers in dairy herds remains unclear. Further work to characterize strains of *S. aureus* isolated from heifers at parturition and from dairy cows is necessary to clarify the importance of different strains of *S. aureus* in the epidemiology of the disease in dairy herds.

The opportunity to study the epidemiology of different strains of *S. aureus* in replacement heifers rose when replacement heifers from the University of Pennsylvania's (UPa) Marshak dairy herd were commingled with replacement heifers from the University of Delaware (UDel) at Rutgers University in the fall of 2004. Only heifers from the UDel and the UPa are commingled at Rutgers University. The historical prevalence of *S. aureus* in cows from the UPa was high, while the prevalence of *S. aureus* in cows from the UDel was low. The historical prevalence of *S. aureus* IMI in primiparous heifers at parturition at the UPa was approximately 10-15%, while unknown but thought to be low at the UDel. A major biosecurity concern was whether heifers from the UPa could serve as fomites of *S. aureus* when commingled with the UDel heifers and thereby create a reservoir of *S. aureus* in the heifers from the UDel. The potential for a newly acquired highly transmissible strain of *S. aureus* to cause an outbreak of mastitis in lactating cows was previously documented.[19]

The objective of this study was to characterize *S. aureus* strains from heifers and cows from the UPa and the UDel, and assess the transmission of *S. aureus* strains between the two respective herds in replacement heifers after commingling at Rutgers University.

Materials and Methods

The UPa and the UDel began to raise their three to four-month-old Holstein heifers born after July 14, 2004 together at Rutgers University. Heifers from both universities were reared away from the milking herd in individual calf hutches, fed milk replacer, and weaned at approximately two months of age. After weaning, heifers from UPa were housed in groups of four calves in super hutches and heifers from UDel were housed in groups of four to six calves in a calf barn. On a bimonthly basis, three to four-month-old heifers from each university were transported and commingled in groups of six or seven heifers of similar age in pens at the Rutgers University calf barn. The naturally ventilated calf barn at Rutgers has ten (12 ft x 25 ft; 3.7 m x 7.6 m) pens on either side of a center drive-through feeding lane. The backs of the pens are cleaned prior to the arrival of new calves, bedded with straw on a daily basis, and cleaned completely every six weeks. The front of each pen has headlocks and a concrete base from which manure is scraped daily and loaded directly into a manure spreader. The heifers have free choice access to a corn silage-based total mixed ration. During the fly season, heifers are treated twice daily by an automatic sprayer with either pyrethrin or permethrin-based insecticides. Heifers are moved to pasture once they are confirmed pregnant. On pasture, heifers pass under a dust bag with insecticide on their way to drink water.

After pregnancy confirmation, heifers were returned to their herd of origin approximately two to seven months before parturition. UPa heifers were returned to the Marshak dairy two to three months after being confirmed pregnant and housed on pasture until two months prior to parturition, when they were moved to the dry cow barn. At the dry cow barn, the heifers were housed with adult dry cows in sawdust-bedded free stalls, and alleys were flushed daily. Cows in the dry cow barn had access to pasture in the summer. During the fly season, heifers, lactating cows, and dry cows were sprayed monthly with a pyrethrin-based insecticide, and various sanitation methods were used to control fly breeding grounds.

UDel heifers were returned to the University of Delaware two to three months prior to parturition and were commingled with adult dry cows in sand free stalls at the dry cow facility. The alleys were scraped on a daily basis, and the cows had access to pasture in the summer. During the fly season, heifers, lactating cows, dry cows, and the premise were sprayed with a pyrethrin or permethrin-based insecticide as needed.

The protocol for this study was approved by UPa's and Rutgers Institutional and Animal Care and Use Committee.

Preliminary cultures from replacement heifers at herd of origin

Teats and muzzles from 185 UPa and 75 UDel weaned heifers three to four months old were screened for *S. aureus* at each dairy prior to transport and commingling at Rutgers University from January 2005 to December 2006. Heifers were not screened for *S. aureus* at Rutgers University. A single swab[a] was used to swab the sides and orifice of each of the heifer's four teats. A second swab was used to sample both inner nares and the muzzle of each heifer. Swabs were direct-plated to Columbia colistin naladixic agar (CNA)[b] and Mannitol salt agar (MSA),[c] and then placed in 10 mL of buffered peptone water (BPW)[d] for enrichment. Plates and BPW were incubated at 95°F (35°C) for 18-24 hours. After incubation, 10 µl of BPW was plated onto CNA and MSA and incubated at 95°F for 18-24 hours. Presumptive *S. aureus* colonies were subcultured for purity on Trypticase soy agar II with 5% sheep blood (TSA)[e] and gram stained. All gram-positive cocci that were catalase and coagulase-positive were considered to be *S. aureus* and included in the study.

Preliminary cultures from cows

One composite milk sample from 129 UPa and 72 UDel milking cows was screened for *S. aureus* in January 2005 before any commingled heifers at the Rutgers facility were returned to their respective herds. All samples were taken by veterinarians from the UPa prior to milking. The teats were pre-wiped with a cloth towel and then pre-dipped with 0.5% iodine. The teats were dried with an individual cloth towel a minimum of 30 seconds after the pre-dip was applied. The teat ends were subsequently scrubbed with alcohol before taking the composite milk samples.

Routine cultures from the milking cows

A composite milk sample from each milking cow from both universities was screened for *S. aureus* three times in 2006 at approximately three-month intervals beginning in February 2006, and four times in 2007 at approximately three-month intervals ending in December 2007. The following groups of cows were considered for analysis:

Group 1. Primiparous cows previously commingled and positive for *S. aureus* at the first routine herd culture after parturition

The prevalence of *S. aureus* in commingled primiparous cows at parturition was estimated as the

number of primiparous cows that had been previously commingled as heifers and were positive for *S. aureus* at the first routine herd culture after parturition, divided by the total number of these primiparous cows. The days from parturition to the first routine herd culture ranged from three to 124. The prevalence of *S. aureus* between the universities was compared using chi square statistic.[f]

Group 2. Primiparous cows previously commingled and negative at parturition for *S. aureus*

The prevalence of *S. aureus* in this group was estimated as the number positive for *S. aureus* on at least one routine culture following negative culture results on the first routine culture after parturition, divided by the number of primiparous cows negative at the first routine culture after parturition. The prevalence of *S. aureus* between the universities was compared using chi square statistic.[f]

Group 3. Primiparous and multiparous cows not commingled as heifers

The prevalence of new infections with *S. aureus* was also estimated at both universities in multiparous cows that were not positive on the baseline screen, as well as primiparous cows that calved over the course of the study and were not commingled as heifers at the Rutgers University heifer facility. In the case of UDel, these would be both primiparous and multiparous cows raised at Rutgers University as heifers prior to the beginning of this study and the commingling with UPa heifers. In the case of UPa, these would be both primiparous and multiparous cows raised at a commercial heifer-rearing facility prior to the beginning of this study and the commingling with UDel heifers at Rutgers University. A new infection was defined as a positive culture for *S. aureus* in a cow at a routine culture time whose previous cultures (preliminary baseline screening or routine culture) were all negative for *S. aureus*. Additionally, in the case of a primiparous cow, a new infection was also defined as a positive culture for *S. aureus* at the first culture period after parturition. The prevalence of *S. aureus* between the universities was compared using chi square statistic.[f]

For Group 2 and 3 prevalence calculations, cows at risk for a new infection were negative for *S. aureus* on all previous cultures, and only one strain from each cow positive for *S. aureus* was used in the calculations.

Milking order

All cows culturing positive for *S. aureus* at UPa were placed in a group designated for *S. aureus* cows and milked last. UDel did not have a separate group designated for cows that cultured positive for *S. aureus*.

Bacteriology

Milk samples were frozen at -4°F (-20°C) for at least 24 hours and subsequently thawed at room temperature. After thawing, 10 µl of each milk sample was plated onto TSA. Plates were incubated at 95°F for 18-24 hours. Presumptive *S. aureus* colonies were subcultured for purity on TSA and gram stained. All gram-positive cocci that were catalase and coagulase-positive were considered coagulase-positive *S. aureus* and included in the study.

Pulsed-field gel electrophoresis

All *S. aureus* isolates were characterized by PFGE using the Centers for Disease Control and Prevention PulseNet™ protocol.[4] Gels were photographed with a Kodak EDAS 290 system[g] and saved as a TIFF file for analysis with BioNumerics software version 5.1.[h] *S. aureus* NCTC 8325 was used as the reference standard for molecular weight determination. Dice coefficients of similarities (S_D) were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages. Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. A similarity coefficient of 0.80 was selected to define pulsed-field profile (PFP) clusters as described previously.[12]

Results

Teats and muzzles

S. aureus was not isolated from 75 UDel heifers, but was isolated from two of 185 (1.1%) heifers at the UPa. One isolate from a muzzle was characterized by PFGE as pulsed field profile 1 (PFP 1; Figure 1), and the second isolate from teat skin was similar to PFP 1 but was isolated only this time. PFP 1 was subsequently identified as the predominant UPa *S. aureus* strain.

Baseline strains of S. aureus

Preliminary culture of composite milk samples established that the UPa and UDel dairy herds had unique baseline strains of *S. aureus* prior to the return of commingled heifers from Rutgers University. The UPa Marshak dairy was categorized as a high-prevalence *S. aureus* herd, and 29 of 129 (22.4%) cows were positive for *S. aureus* on initial culture (Table 1). Three different strains of *S. aureus* were identified. Twenty-six of 29 cows (90%) had the predominant strain designated PFP 1, and two additional strains of *S. aureus* were characterized as PFP 2 (n=2) and PFP 3 (n=1) (Table 1; Figure 1). The UDel was categorized as a low-prevalance *S. aureus* herd, and two of 72 cows (2.7%) each had a unique strain of *S. aureus* that differed from the strains isolated from the UPa herd (Table 1). The two UDel strains were designated as PFP 4 and PFP 5.

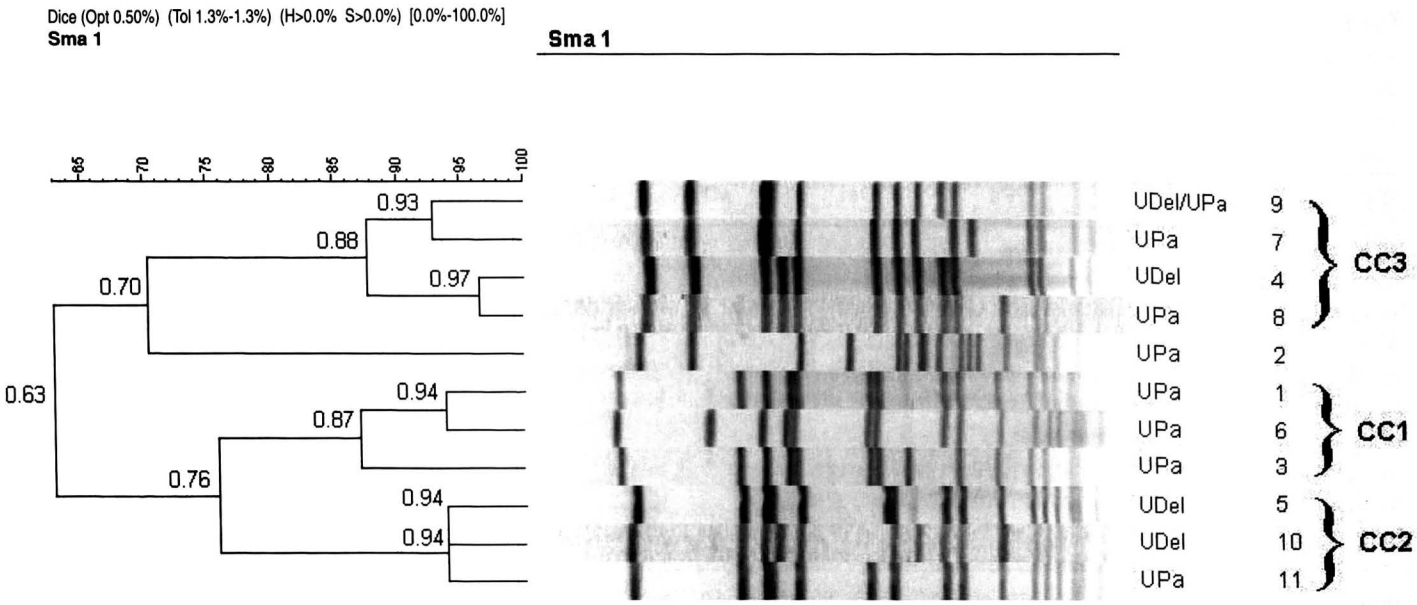

Figure 1. The figure shows the 11 PFPs identified in lactating cows in this study. Dice coefficients of similarity (S_D) are identified on a dendrogram derived from the unweighted pair group method using arithmetic averages (UPGMA). Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. A similarity coefficient of 0.80 was selected to define pulsed-field profile (PFP) clusters, CC1, CC2, and CC3. PFP 2 from the University of Pennsylvania (UPa) was unique and did not belong in any of the clusters identified in this study.

Analysis of the dendrogram and S_D values when PFPs 1 through 5 were compared with each other indicated that, with the exception of PFPs 1 and 3, none of the isolates were clonally related. PFPs 1 and 3 showed a dice coefficient of 0.87 (S_D 0.87), suggesting these two strains are part of a clonal cluster (CC 1) based on a cluster cutoff of 0.80.[12]

Group 1. Primiparous cows previously commingled and positive for *S. aureus* at the first routine herd culture after parturition

The prevalence of *S. aureus* in primiparous cows was similar at both universities (P=0.77; Table 1). The prevalence of *S. aureus* in primiparous cows previously commingled at the UPa was 9% (10/110). The strains were characterized by PFGE as PFP 1 (n=5), PFP 6 (n=1), PFP 7 (n=1), PFP 8 (n=1), and PFP 11 (n=2) (Table 1, Figure 1). Dice coefficients suggested that PFP 6 and PFP 1 were clonally related (S_D 0.94), and the single band difference between these two strains suggests a DNA insertion event.[21] This strain was isolated only at the UPa and with greater frequency over time (Table 1). PFP 11 belonged to a closely related clonal cluster (CC 2), but with four band differences compared with PFP 1 it is unlikely that PFP 11 and PFP 1 are epidemiologically related. PFPs 7 and 8 were also determined to be clonally related (S_D 0.88), but belonged to a different clonal cluster (CC 3) than PFPs 1, 6, and 11 (Figure 1).

The prevalence of *S. aureus* in primiparous cows previously commingled from the UDel was 7.7% (4/52; Table 1). The strains were characterized by PFGE as PFP 9 (n=3) and PFP 10 (n=1) (Table 1, Figure 1). PFP 9 was part of CC 3 and showed a dice coefficient of 0.93 to PFP 7 isolated from a single cow at the UPa. PFP 10 was part of CC 2 and showed a dice coefficient of 0.94 to PFP 11 isolated at the UPa.

Group 2. Primiparous cows previously commingled and negative at parturition for *S. aureus*

The UPa had significantly (P=0.03) more infections than the UDel in primiparous cows that were previously commingled and that were negative for *S. aureus* at the first routine culture after parturition (Table 1). Nine of 100 (9.0%) cows cultured positive for *S. aureus* on at least one routine culture following negative culture results for *S. aureus* at the first routine herd culture after parturition. The strains were characterized by PFGE as PFP 1 (n=3), PFP 6 (n=5), and PFP 11 (n=1) (Table 1, Figure 1). In total, 19/110 (17%) primiparous cows from the UPa that had been previously commingled cultured positive for *S. aureus* at least once during the routine cultures. There were no primiparous cows previously commingled from the UDel that cultured positive for *S. aureus* on a routine culture after the first routine herd culture after parturition. In total, 4/52 (7.7%) primiparous cows commingled from the UDel cultured positive for *S. aureus* at least one time during the routine cultures.

Table 1. Pulsed-field profile strains of *Staphylococcus aureus* isolated at routine culture times.

	Preliminary cultures[a]		Routine cultures from the milking cows[b]					
	Baseline strains		Group 1[c]		Group 2[d]		Group3[e]	
PFP strains	UPa	UDel	UPa	UDel	UPa	UDel	UPa	UDel
1	26		5		3		22	
2	2							
3	1						1	
4		1						
5		1						
6			1		5		7	
7			1					
8			1					
9				3			1	1
10				1				2
11			2		1			
Total strains	29	2	10	4	9	0	31	3
Cows at risk[f]	129	72	110	52	100	48	162	124
Baseline prevalence	22.4%	2.7%						
Prevalence chi square			9%	7.7% P=0.77	9.0%	0% P=0.03	19%	2.4% P<0.0001

[a]One composite milk sample taken in January 2005 from each milking cow from the University of Pennsylvania (UPa) and the University of Delaware (UDel) prior to commingling heifers at Rutgers University.
[b]Composite milk samples taken three times in 2006 and four times in 2007 from each primiparous and multiparous milking cow from UPa and UDel.
[c]Primiparous cows previously commingled and positive for *S. aureus* at the first routine herd culture after parturition.
[d]Primiparous cows previously commingled, negative at parturition for *S. aureus*, and positive for *S. aureus* on a subsequent routine culture.
[e]Primiparous and multiparous cows not commingled as heifers at Rutgers University.
[f]Denominator used in prevalence calculation. For Group 1, cows at risk were all the UPa and UDel commingled primiparous cows sampled in the study. For Groups 2 and 3, cows at risk were negative for *S. aureus* on all previous cultures.

Group 3. Culture results from primiparous and multiparous cows not commingled as heifers

The UPa had significantly more infections in this group of cows than the UDel (P<0.0001; Table 1). Thirty-one new infections with *S. aureus* were recorded from 162 cows at risk at the UPa, and three new infections were recorded from 124 cows at risk at the UDel. The predominant strain of *S. aureus* isolated during the baseline screen (PFP 1) remained the predominant strain isolated by routine culture from cows that were not positive for *S. aureus* at the initial screen (Table 1). Isolates that showed PFP 6 was the next most common strain observed in this group. Two strains were characterized by PFGE as PFP 3 and PFP 9, respectively. These two strains were unrelated (S_D 0.63) and belonged to different clonal clusters (CC 1 and CC 3, respectively). However, PFP 3 and PFP 1 were very closely related (S_D 0.87) and showed only two band differences. PFP 9 was isolated from a purchased primiparous cow from the UPa that had not previously been commingled. PFP 9 was also seen in three commingled primiparous cows and one cow that had not been previously commingled from the UDel. During the course of this study, four heifers had been purchased by the UPa. All four heifers were cultured at parturition, and one heifer had a strain isolated that was characterized by PFGE as PFP 9 that had also been identified from a UDel cow before any commingled heifers were returned to the herds.

Discussion

The UPa had a high historical prevalence of mastitis caused by *S. aureus*. Preliminary microbiology culture and strain characterization by PFGE confirmed

this, and determined that the baseline prevalence was 22.4% and that a single strain of *S. aureus* (PFP 1) predominated. The UDel was designated as a low-prevalence herd and results confirmed this, and determined the baseline prevalence of *S. aureus* as 2.7%. Characterization by PFGE showed that the *S. aureus* strains isolated were genetically heterogeneous. Analysis of the dendrogram and S_D values when PFPs 1 through 5 were compared with each other indicated that, with the exception of PFPs 1 and 3, none of the isolates were clonally related. Clones are isolates that are indistinguishable from each other by a variety of genetic tests or are so similar that they are presumed to be derived from a common parent.[21]

The predominant strain of *S. aureus* (PFP 1) at the UPa was not spread to the UDel through commingling heifers at the Rutgers heifer-rearing facility. Although the prevalence of *S. aureus* in cows from the UPa was high compared to the UDel, the prevalence of *S. aureus* at parturition in primiparous cows previously commingled was similar, suggesting that factors other than, or in addition to, the prevalence of *S. aureus* in the herd contribute to the prevalence of *S. aureus* in primiparous cows at parturition. These results are in agreement with Roberson *et al*[16] who showed that the prevalence of coagulase-positive *Staphylococcus* in primiparous cows at parturition was similar whether they came from a high- or low-coagulase-positive *Staphylococcus* herd. Our results should be interpreted with caution since the first milk samples in this study were taken at routine culture times (3-124 days) instead of before the first milking after parturition, so primiparous cows could have been infected during milking. Although sampling schemes varied in the literature as to the number of days after parturition when primiparous cows were cultured, our results are similar to the data of Roberson *et al*[18] and Middleton *et al*[13] in that the majority of *S. aureus* strains that caused mastitis in primiparous cows at parturition were also isolated from the lactating herd mammary glands or body sites on the heifers. In our study, 60% of the primiparous cows from the UPa at parturition were infected with strains also found in the lactating cows compared to 70% in the Roberson *et al*[18] study and 71% in the Middleton *et al*[13] study.

Nine previously commingled primiparous cows from the UPa acquired strains of *S. aureus* after the first routine herd culture after parturition, compared with zero cows at the UDel. Eight out of nine of these cows had strains either identical or closely related to the predominant strain (PFP 1), suggesting that the overall prevalence and strain types within a lactating herd significantly contributes to the overall new infection rate in primiparous cows in later lactation or lactations. These results are in agreement with Tenhagen *et al*[20] who found that strains acquired in later lactation by primiparous cows compared to those found at parturition originated from the lactating cow. This study showed that in both commingled primiparous cows and cows not commingled the predominant endemic strain of *S. aureus* isolated during baseline studies at the UPa was still the predominant strain isolated at the end of the study. A second strain (PFP 6) increased in prominence at the UPa in the latter part of the study, and although evidence suggests that PFP 6 may have evolved from PFP 1, the significance of this strain is currently unknown. Together, these two strains made up 86% of the strains isolated after the baseline screen (Table 2). One additional strain (PFP 3), isolated from one cow from the UPa, was part of CC 1. These results would agree with Joo *et al*[7] who reported that herds tend to have a predominant unique strain of *S. aureus*, and with Tenhagen *et al*[20] who showed that the majority of isolates from high-prevalence *S. aureus* herds belonged to one or two strains, but other less prevalent strains could also be isolated in all herds.

The two strains of *S. aureus* isolated during the baseline screen from the UDel (PFPs 4 and 5) were not isolated again from routine cultures from cows not commingled as heifers. The remaining strains isolated from the UDel (PFPs 9 and 10) were from two different clonal clusters (CC 3 and CC 2, respectively) that were not closely related (S_D 0.63). A total of seven UDel cows (four primiparous commingled and three cows not commingled) had *S. aureus* isolated. None of the UDel strains were closely related to PFP 1. Some of the UDel strains and non-predominant strains isolated from the UPa were part of the same clonal clusters

Table 2. Total pulse-field profile (PFP) strains of *Staphylococcus aureus* isolated at routine culture times from all cows not positive at the baseline screening (Groups 1, 2, and 3).

PFP strains	UPa[a]	UDel[b]
1	30	
2		
3	1	
4		
5		
6	13	
7	1	
8	1	
9	1	4
10		3
11	3	
Totals	50	7

[a]UPa is the University of Pennsylvania.
[b]UDel is the University of Delaware.

(Figure 1). These findings are more likely normal occurrences, since there is not strong evidence in this study to support transmission of *S. aureus* strains between the universities. Middleton *et al*[13] found 82% of *S. aureus* strain-types were unique to one herd, with 12% of strains occurring in two herds, while Joo *et al*[7] found 66% of *S. aureus* strains occurred in a single herd, 27% of strains occurred in two herds, and 8% occurred in three herds.

In our study, three to four-month-old commingled heifers returned to their herds of origin at least two to seven months before parturition were more at risk of acquiring their own herd's endemic strains of *S. aureus* rather than strains they were potentially exposed to at the Rutgers heifer-rearing facility. Unfortunately, in this study the potential risk of transmission of *S. aureus* strains between heifers at the heifer facility would have to be characterized as low since there was a low prevalence of *S. aureus* found in the replacement heifers sampled in the herds of origin prior to commingling. In addition, heifers were not sampled at Rutgers University.

Although there was some diversity of strains of *S. aureus* identified from the UPa commingled primiparous cows at the first routine culture following parturition, these strains were not closely related to the predominant strain. Overwhelmingly, both commingled primiparous cows negative at parturition for *S. aureus* (Group 2) and cows not infected at the baseline screen (Group 3) became infected in later lactation or lactations with the predominant strain of *S. aureus*. The data would support that low-prevalent strains not found in the lactating herd and isolated from commingled primiparous cows at parturition from the UPa were not of major importance, as they were not prevalent in later lactation or lactations. Although 7.7% of commingled UDel primiparous cows calved with *S. aureus*, as identified at the first routine culture, none of the remaining commingled primiparous cows developed further *S. aureus* infections, and only three cows not initially screened (Group 3) developed *S. aureus* infections. Results of this study support that UPa strains of *S. aureus* were not spread to UDel heifers through commingling heifers at the Rutgers heifer facility. Determining the origin and significance of colonization of *S. aureus* in the primiparous cows at UDel was beyond the scope of this study. However, these results support conclusions from a previous study that replacement heifers could serve as a reservoir of *S. aureus* even on dairies with a low prevalence of *S. aureus* in the lactating herd.[17]

Roberson *et al*[17] reported wide ranges for the isolation of *S. aureus* on at least one body site from preweaned heifers, ranging from 0% from low *S. aureus*-prevalence herds to 50% from high *S. aureus*-prevalence herds. Growing heifers that were four to 12 months old had the lowest prevalence of *S. aureus* isolated; this figure ranged from 4.3% to 14% in high-prevalence *S. aureus* herds.[17] In our study, *S. aureus* was isolated from growing heifers of three to four months of age in 1% of the UPa heifers and 0% from UDel heifers. We sampled the muzzle, teat skin, and teat orifice because these were the most common sites found to be colonized with *S. aureus* in the study by Roberson *et al*.[17] However, we sampled heifers only one time compared with Roberson *et al*[17] who sampled four body sites of heifers five times each. As a result, the risk of heifers acquiring unique *S. aureus* strains from other commingled herds could vary, depending on the age they were commingled and also the management practices of the herds whose heifers are commingled together. Middleton *et al*[13] reported that herds in which replacement heifers were purchased had a higher prevalence of *S. aureus* mastitis than herds in which lactating cattle were purchased.

Results from our study highlight the known impact that a high prevalence of *S. aureus* infection in a lactating herd can have on the new infection rate within the lactating herd. It also highlights the effect in primiparous cows at parturition, and is in agreement with other studies that support the infected mammary glands of lactating cows as the major reservoir of *S. aureus* on the farm.[13,18,20] Further studies should focus on designing strategies to prevent infection in primiparous cows at parturition with lactating cow (endemic) strains, as these strains could be more significant than other strains isolated at parturition. The impact of management practices such as feeding milk replacer versus whole milk, location of replacement heifers in relation to the milking herd, housing of replacement heifers prior to calving, and fly control on the epidemiology of *S. aureus* infections in replacement heifers needs further study.

In agreement with Larsen *et al*,[9] results from this study would support the hypothesis that the initial predominant strain at the UPa (PFP 1) was endemic, contagious, and pathogenic. Likely, this strain had characteristics that enabled it to evade important host mechanisms of defense and establish intramammary infections more successfully than the other less prevalent types of strains isolated in this study.[1] Although the UPa's endemic strain of *S. aureus* was not transmitted to the UDel through commingling heifers at a heifer-rearing facility, dairy herds should be made aware that some strains of *S. aureus* are more pathogenic and at a minimum, commingled or purchased replacement heifers should be screened for the presence of *S. aureus* prior to commingling with the lactating herd so that effective control measures can be established.

Conclusion

In this study, three to four-month-old commingled heifers returned to their herd of origin two to seven

months prior to parturition were more at risk of acquiring an endemic strain of *S. aureus* rather than strains they may have potentially been exposed to at a commercial heifer-rearing facility. In this study, low-prevalent strains not found in the lactating herd and isolated from primiparous cows at parturition were of minor importance, and the infected mammary glands of lactating cows were the major reservoir of *S. aureus*.

Endnotes

[a]BBL™ Culture Swab, Becton Dickinson, Sparks, MD
[b]Columbia colistin naladixic agar, Becton Dickinson, Sparks, MD
[c]Mannitol salt agar, Becton Dickinson, Sparks, MD
[d]Buffered peptone water, Oxoid, Ltd. Basingstoke, Hampshire, England
[e]Trypticase soy agar II, Becton Dickinson, Sparks, MD
[f]Statistix Analytical Software, version 3.5, Tallahassee, FL
[g]Kodak EDAS 290 system, Eastman Kodak Co, New Haven, CT
[h]BioNumerics software version 5.1, Applied Maths, Kortrijk, Belgium

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