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Use of pulsed-field gel electrophoresis for detecting differences in *Staphylococcus aureus* strain populations between dairy herds with different cattle importation practices

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SUMMARY

The hypothesis tested was that dairy herds which import cattle for replacement or expansion have a higher prevalence of *Staphylococcus aureus* mastitis and a greater number of new *Staphylococcus aureus* strains enter their herds than closed herds. Fifteen commercial dairy herds were divided into four groups based on cattle importation practices. Composite foremilk samples were collected at 4-monthly intervals for 1 year from all lactating cattle. Additionally, foremilk samples were collected from cattle at parturition and skin swabs were taken from the udder of primiparous heifers. All samples were cultured for *Staphylococcus aureus* and isolates were strain-typed using pulsed-field gel electrophoresis. Herds that purchased replacement heifers had a higher prevalence of *Staphylococcus aureus* mastitis than herds that purchased lactating cattle for expansion ($P = 0{\cdot}02$). Herds that purchased replacement heifers had more total strains of *Staphylococcus aureus* ($P = 0{\cdot}01$) and more new strains ($P = 0{\cdot}04$) enter the herd than closed herds.

INTRODUCTION

In recent years the demographics of the Pacific Northwest dairy industry have been undergoing considerable change. The number of herds is decreasing, while herd size is increasing. Hence, herds must be undergoing expansion with outside cattle. The National Animal Health Monitoring System (NAHMS) reported in 1996 that nearly half of United States dairy operations added animals to their herds [1]. The majority of those additions were as bred heifers and lactating cows. Importing cattle into a dairy herd presents a threat to herd biosecurity, and therefore *Staphylococcus aureus*, the most prevalent contagious mastitis pathogen [2, 3], has the potential to be carried into a herd by imported cattle.

While importing lactating cattle with *S. aureus* intramammary infection (IMI) presents an obvious risk for mastitis in uninfected resident cattle, a less obvious risk is the replacement heifer. Roberson et al. [4] reported that up to one-third of heifers may have a coagulase-positive staphylococcal IMI at parturition. Routine contagious mastitis pathogen control procedures as outlined by Neave et al. [5] may afford some protection against the introduction of new *S. aureus* mastitis strains into a herd. However, Smith and co-workers [6] reported an outbreak of mastitis caused by a single strain of *S. aureus* in the face of routine contagious mastitis control procedures. The outbreak strain was highly transmissible and caused a

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peak prevalence of *S. aureus* IMI greater than 22% in a herd with a historical prevalence of less than 3%. Therefore, the hypothesis tested in the present study was that dairy herds which incorporate cattle raised off the premises (import cattle) will experience an increased prevalence of *S. aureus* IMI and have a greater number of new *S. aureus* strains enter their herds than closed herds that rear their own replacements.

MATERIALS AND METHODS

Fifteen commercial dairy herds located in the Pacific Northwest were enrolled in the study. Herds were divided into four groups based on cattle importation practices. The groups were (I) herds that contract reared replacement heifers ($n = 4$); (II) herds that purchased replacement heifers ($n = 3$); (III) herds undergoing expansion with recently purchased lactating cattle ($n = 2$); and (IV) closed herds that reared their own replacements (control) ($n = 6$).

Composite foremilk samples were collected from all lactating cattle in each herd at approximately four-monthly intervals for 1 year (sample periods = 3). Additionally, composite foremilk samples were collected from cows and heifers at the time of parturition. Lactating cattle purchased for expansion had composite foremilk samples collected prior to entering a study herd. All milk samples were aseptically collected using established guidelines and stored at −20 °C [7]. Milk samples were cultured for bacterial mastitis pathogens using standardized procedures [7]. Briefly, milk samples were thawed at room temperature (25 °C) and 50 μl aliquots were cultured on Columbia agar with 5% sheep blood (Remel, Lenexa, KS, USA). Cultures were incubated for 24 h at 37 °C. Cultures were read at 24 and 48 h. Staphylococci were presumptively identified based on colonial morphology and haemolytic patterns. All suspect staphylococci were further identified using the tube coagulase test [7]. Coagulase-positive staphylococci were differentiated using a commercial biochemical typing system (api Staph, bioMerieux, Inc., Hazelwood, MO, USA). All *S. aureus* isolates were stored in brain heart infusion (BHI) broth (Difco, Becton Dickinson and Company, Sparks, MD, USA) at −20 °C until further analysis.

Teat and udder skin swabs were collected from imported heifers upon arrival at the farm, but prior to the heifers being co-mingled with resident cattle. Likewise, teat and udder skin swabs were collected from heifers in control herds 1–2 months prior to parturition. The barrel and tip of all four teats and the inguinal region of each heifer were swabbed using sterile cotton-tipped swabs soaked in Vogel–Johnson (V–J) media [8]. All swabs were subsequently placed in V–J media and stored at −20 °C until further analysis. Teat and udder skin swabbing solutions were thawed, pre-incubated at 37 °C for 4 h, and cultured on both Columbia agar with 5% sheep blood (Remel, Lenexa, KS, USA) and modified Baird–Parker (MBP) agar [9]. Blood agar plates were read at 24 and 48 h and MBP plates were read at 48 h. Staphylococci were presumptively identified based on colonial morphology and haemolytic patterns on blood agar and the presence of small, shiny black colonies on MBP agar. Suspect staphylococci were further differentiated as described previously for milk. All *S. aureus* isolates were stored in BHI at −20 °C until further analysis.

Staphylococcus aureus isolates were strain-typed using the pulsed-field gel electrophoresis (PFGE) technique described by Joo and co-workers [10] with modification. Two PFGE units were used in the present study. The majority of isolates were strain-typed using the CHEF DRII (Bio-Rad Laboratories, Hercules, CA, USA) and a minority of isolates were run on the HG 1000 HULA Gel unit (Hoefer Scientific Instruments, San Francisco, CA, USA). Bacterial growth conditions, agarose plug manufacture, and DNA digestion with *Sma*I (*Sma*I, Gibco–BRL, catalogue number 15228-018, Life Technologies, Gaithersburg, MD, USA) were performed as described by Joo and co-workers [10]. Digests of staphylococcal DNA were separated by PFGE using a 1% agarose gel (Pulsed Field Certified Agarose, catalogue number 162-0137, Bio-Rad Laboratories, immersed in 0·5% buffer (Bio-Rad 10× Tris/boric acid/EDTA buffer, catalogue number 161-0733, Bio Rad Laboratories) at 14 °C for 20 h. Pulsed-field gel electrophoresis unit settings were 6 V/cm with a 130° ramp angle and a 5–50 s pulse time for the CHEF DRII and 200 V continuous with a 130° ramp angle and a 5–50 s switch time for the HG 1000 HULA Gel unit. A lambda ladder molecular weight size standard was run in the first and last lane of each gel (CHEF DNA Size Standards, catalogue number 170-3635, Bio-Rad Laboratories). The distance between fragments (bands) on the lambda ladder was 48·5 kb with the smallest fragment being 48·5 kb. Molecular weights of the nucleic acid fragments of unknowns were determined using Pro-RFLP software (DNA ProScan, Inc., Nashville, TN, USA). Isolates were

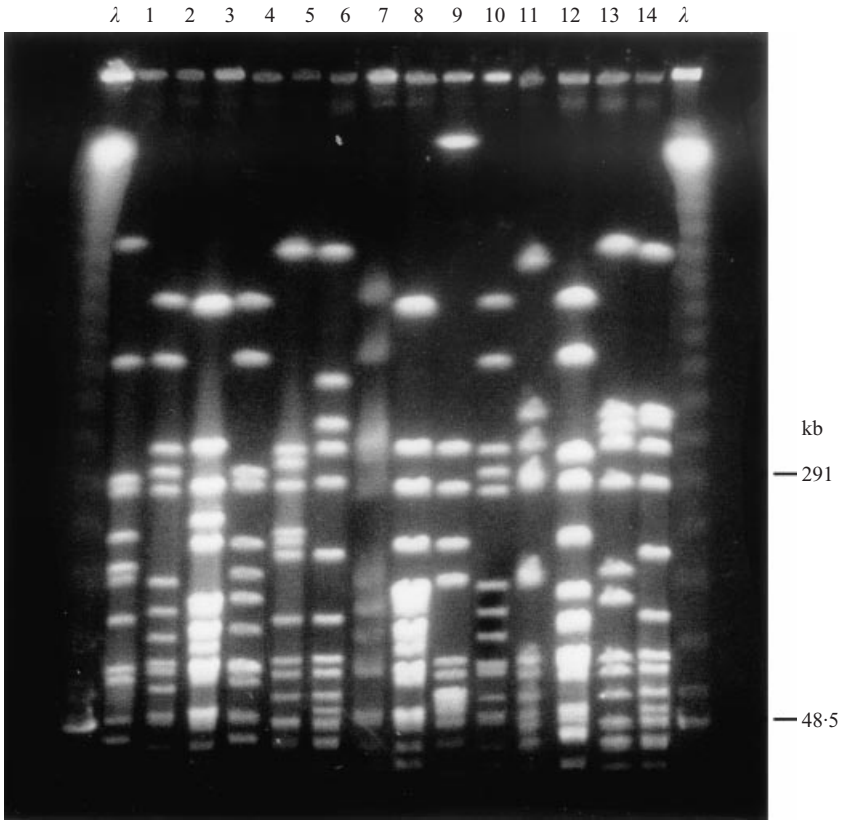

Fig. 1. Photograph of the results of PFGE showing representative *Staphylococcus aureus* isolates from each herd. The first and last lanes represent lambda ladder size standards. Lanes labelled 1–14 contain *Sma*I digests of DNA from individual *S. aureus* isolates. One isolate from each herd with *S. aureus* isolated is represented. Note that each isolate is unique from the next, i.e. no isolate has exactly the same number of bands of like molecular weights, and therefore 14 strain-types are represented.

initially grouped using Cluster Analysis (SAS Institute, Cary, NC, USA). The variables considered were the number and size of the electrophoretically separated nucleic acid fragments. A dendrogram was created from the Cluster Analysis and isolates differing by greater than 0·1 Euclidean units were assigned to different groups. Visual examination of the banding pattern of representative isolates from each group was used to confirm group identification (Fig. 1).

Prevalence of *S. aureus* infections was calculated by dividing the number of cows with *S. aureus* infections by the total number of cows sampled in a given sample period for each herd. Herds were grouped according to cattle importation practices. Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks was used to determine differences between groups with regard to prevalence of *S. aureus* IMI (Model: Prevalence of *S. aureus* IMI = Group + Error) (NCSS 97, NCSS Statistical Software, Kaysville, UT, USA). Sample period was not entered into the model because a sample period effect was not expected due to the continuous entry of outside cattle in groups I and II. With regard to seasonal effects on mastitis, Table 1 shows that *S. aureus* mastitis prevalence remained relatively constant across all three sample periods in each group. There was the potential for a sample period effect in group III because these two herds underwent expansion with cattle from another herd in the middle of the study. However, the χ^2 test showed no significant difference between the proportion of cattle with *S. aureus* IMI at the beginning and end of the study in either herd in group III ($P = 0{\cdot}44$, $0{\cdot}21$, respectively). Hence, all sample periods within herd were averaged. Kruskal–Wallis one-way ANOVA on ranks was used to detect differences in *S. aureus* prevalence on teat and udder skin between groups ($P = 0{\cdot}05$) (Model: *S. aureus* teat and udder skin prevalence = Group + Error). Similarly, Kruskal–Wallis one-way ANOVA on ranks was used to detect differences in the prevalence of *S. aureus* IMI in heifers or cows at parturition between groups ($P = 0{\cdot}05$) (Model: *S. aureus* IMI prevalence in heifers or cows, respectively, at parturition = Group + Error).

Table 1. *The mean* S. aureus *IMI prevalence data by group and sample period* (%). *Group designations are as follows:* (*I*) *Contract reared replacement heifers;* (*II*) *purchased replacement heifers;* (*III*) *purchased lactating cattle for expansion,* (*IV*) *closed herds that reared their own replacement stock*

	Prevalence of *Staphylococcus aureus* IMI			
	Sample period (%)			
Group	1	2	3	Overall
I				
Mean	2·3	1·0	1·7	1·7
S.E.*	0·5	0·4	0·6	0·8
II				
Mean	4·9	5·1	4·6	4·9†
S.E.	3·2	2·9	2·7	0·9
III				
Mean	0·6	0·7	0·7	0·6
S.E.	0·6	0·2	0·0	1·1
IV				
Mean	2·8	3·0	2·1	2·6
S.E.	0·9	1·1	0·8	0·6

* S.E., Standard error of the mean.
† Kruskal–Wallis one-way ANOVA on ranks: group II herds had a greater prevalence of *S. aureus* IMI than group III herds ($P = 0{\cdot}02$).

The total number of strains of *S. aureus* isolated from each herd was calculated and one-way ANOVA was used to detect differences in the number of *S. aureus* strain-types per herd between groups ($P = 0{\cdot}05$) (Model: Number of *S. aureus* strain-types = Group + Error) (NCSS 97, NCSS Statistical Software). Similarly, the number of new strains of *S. aureus* isolated since the initial sampling (sample period 1) in each herd was calculated and one-way ANOVA was used to detect differences in the number of new strains per herd between groups ($P = 0{\cdot}05$) (Model: Number of new strain-types of *S. aureus* = Group + Error). Finally, the number of strains shared with at least one other herd was calculated for each herd and one-way ANOVA was used to detect differences in the number of shared strains of *S. aureus* per herd between groups ($P = 0{\cdot}05$) (Model: Number of *S. aureus* strains shared by at least one other herd = Group + Error). When differences were detected using the Kruskal–Wallis one-way ANOVA on ranks the Kruskal–Wallis multiple comparison *Z*-value test was used to detect which groups differed from each other (NCSS 97, NCSS Statistical Software). When differences were detected with one-way ANOVA Duncan's multiple-comparison test was used to detect which groups differed from each other ($P = 0{\cdot}05$) (NCSS 97, NCSS Statistical Software).

RESULTS

Staphylococcus aureus was isolated from 358 cattle with 330 cattle contributing isolates from mammary gland secretions and 28 cattle contributing isolates from teat and udder skin. *Staphylococcus aureus* isolates from 323 of these cattle were strain-typed by PFGE with 296 cattle contributing isolates from mammary gland secretions and 27 cattle contributing isolates from teat and udder skin. Isolates from the remaining 35 cattle could not be grown from BHI storage media after long term storage at −20 °C. Prevalence of *S. aureus* IMI in individual herds ranged from 0·0 to 11·4% with 14 of the 15 (93%) herds having at least one cow with a *S. aureus* IMI. Mean prevalence of *S. aureus* IMI within group at each sampling is shown in Table 1. Teat and udder skin swab data was only collected from 12 of the 15 (80%) herds. Prevalence of *S. aureus* on the teat and udder skin of heifers ranged from 0·0 to 17·1% with only 3 of the 12 (25%) herds having cattle with *S. aureus* on their teat and udder skin (Table 2). Prevalence of *S. aureus* IMI in heifers at parturition ranged from 0·0 to 12·3% and prevalence of *S. aureus* IMI in cows at parturition ranged from 0·0 to 5·7% (Table 3). Kruskal–Wallis one-way ANOVA on ranks showed significant differences between groups with regard to *S. aureus* IMI prevalence ($P = 0{\cdot}02$). The Kruskal–Wallis multiple-comparison *Z*-value test showed that group II differed from group III, i.e. herds that purchased replacement heifers had a greater prevalence of *S. aureus* IMI than herds that purchased lactating cattle for expansion. No statistical difference was found between groups with regard to prevalence of *S. aureus* on the teat and udder skin of heifers prior to parturition ($P = 0{\cdot}54$). Likewise, no significant difference was found between groups with regard to the prevalence of *S. aureus* IMI at parturition for heifers or cows ($P = 0{\cdot}44$, 0·09, respectively). In group III (herds purchasing lactating cattle for expansion; $n = 2$) samples were only available from purchased cattle in one herd (herd 9). Herd 9 underwent a herd merger with a larger herd. Composite foremilk samples were collected from all lactating cattle shortly before the purchased herd merged with herd 9. Only

Table 2. *The number of teat/udder skin swabs collected within herd and group and the proportion that yielded* Staphylococcus aureus

		Heifer skin swab samples					Heifer skin swab samples		
Group	Herd	*S. aureus*-positive	No.†	Percent* positive	Group	Herd	*S. aureus*-positive	No.	Percent* positive
I	1	0	66	0·0	III	9	NA‡	NA	NA
	2	0	48	0·0		15	3	56	5·4
	5	0	51	0·0					
	14	12	70	17·1					
			Mean	4·3				Mean	5·4
			S.E.	3·2				S.E.	6·5
II	7	NA	NA	NA	IV	4	NA	NA	NA
	10	0	7	0·0		6	0	0	0·0
	16	13	85	15·3		8	0	4	0·0
						11	0	1	0·0
						12	0	44	0·0
						13	0	54	0·0
			Mean	7·6				Mean	0·0
			S.E.	4·6				S.E.	2·9

* Kruskal–Wallis one-way ANOVA on ranks showed no significant differences between groups ($P = 0{\cdot}54$).
† Number of samples collected.
‡ NA, No samples available for analysis.
S.E., Standard error of the mean.

one cow in the herd that merged with herd 9 had a *S. aureus* IMI. The PFGE strain-type of the *S. aureus* isolate was unique to the merged herd and was not isolated again following the herd merger nor was it isolated from cattle in any other herd studied.

PFGE strain-typing revealed 76 strains of *S. aureus*. The number of strains per herd ranged from 0 to 20 strains (Table 4). Fourteen of the 76 (18%) *S. aureus* strain-types were found in more than one herd. Of the *S. aureus* strains shared by at least 1 other herd, 9 strains were found in 2 herds, 1 strain was found in 3 herds, and 4 strains were found in greater than 3 herds. Thirteen of the 15 (87%) herds had at least one strain of *S. aureus* that was shared by another herd. At least one new strain of *S. aureus* emerged in 12 of the 15 (80%) of the herds. The three herds that did not acquire new strains of *S. aureus* were in group IV (closed herds). One-way ANOVA for total number strains showed a significant difference between groups ($P = 0{\cdot}01$). Duncan's multiple-comparison test showed that group II herds (herds purchasing replacement heifers) had a greater number of *S. aureus* strains than group IV herds (closed herds). Similarly, there was a significant difference between groups with regard to the number of new strains of *S. aureus* occurring after the initial sampling (sample period 1) ($P = 0{\cdot}04$). Duncan's multiple comparison test showed that group II herds acquired a greater number of new strains of *S. aureus* than group IV herds. No significant difference was found for the number of strains of *S. aureus* shared by at least one other herd between groups ($P = 0{\cdot}10$). In the three herds where *S. aureus* was found on the teat skin pre-partum, the strains found on the teat and udder skin were the same as strains isolated from the milk of lactating cattle 67% of the time. Similarly, 47% of the strains found on teat and udder skin were the same as strains isolated from heifer mammary secretions. Strains isolated from mammary secretions of heifers at parturition were the same as strains isolated from the milk of lactating cattle 71% of the time, whereas strains isolated from cows at parturition were the same as strains isolated from the milk of lactating cattle 67% of the time. The PFGE electrophoretic banding patterns for selected isolates from each herd are shown in Figure 1. The first and last lanes, labelled λ, represent lambda ladder size standards as described above. Lanes labelled 1–14 show the banding pattern of nucleic acid fragments produced by *Sma*I digestion of *S. aureus* DNA from representative isolates from each herd. Each *S. aureus* isolate shown is unique in that none of the isolates have identical numbers of bands of like molecular weights. Hence, 14 strains of *S. aureus*, one from each herd (no *S.*

Table 3. *The proportion of cows and heifers with* Staphylococcus aureus *intramammary infection at parturition*

		Heifers			Cows		
Group	Herd	*S. aureus*-positive	No.*	Percent† positive	*S. aureus*-positive	No.	Percent‡ positive
I	1	1	105	1·0	0	155	0·0
	2	0	15	6·7	2	185	1·1
	5	0	76	0·0	1	165	0·6
	14	7	63	11·1	0	268	0·0
			Mean	4·7		Mean	0·4
			S.E.	2·0		S.E.	0·7
II	7	0	16	0·0	3	61	4·9
	10	5	104	4·8	3	53	5·7
	16	20	162	12·3	11	362	3·0
			Mean	5·7		Mean	4·5
			S.E.	2·3		S.E.	0·8
III	9	0	15	0·0	1	104	1·0
	15	4	165	2·4	1	288	0·3
			Mean	1·2		Mean	0·7
			S.E.	2·8		S.E.	0·9
IV	4	0	34	0·0	0	50	0·0
	6	0	10	0·0	0	8	0·0
	8	0	2	0·0	0	17	0·0
	11	0	3	0·0	0	61	0·0
	12	3	70	4·3	1	123	0·8
	13	1	68	1·5	5	117	4·3
			Mean	1·0		Mean	0·8
			S.E.	1·6		S.E.	0·5

* Number of samples collected.
† Kruskal–Wallis one-way ANOVA on ranks showed no significant differences between groups for heifers at parturition ($P = 0{\cdot}44$).
‡ Kruskal–Wallis one-way ANOVA on ranks showed no significant differences between groups for cows at parturition ($P = 0{\cdot}09$).
S.E., Standard error of the mean.

aureus were isolated from herd 4), are represented. *Staphylococcus aureus* strain data are summarized in Table 4.

DISCUSSION

The use of PFGE to differentiate *S. aureus* isolated from the bovine udder into strain-types has been demonstrated previously [10, 12]. Similarities to those previous studies with regard to the distribution of *S. aureus* strains within and among herds were found in the present study. However, no study to date has used molecular fingerprinting to assess the effect of cattle importation practices on the number of strains of *S. aureus* that may occur in a dairy herd. The main objective of the present study was to test the hypothesis that dairy herds which incorporate cattle raised off the premises will experience an increased prevalence of *S. aureus* IMI and have a greater number of new *S. aureus* strains than closed herds that rear their own replacements. The data presented partially support this hypothesis. Herds that purchased replacement heifers had a higher prevalence of *S. aureus* IMI than herds that purchased lactating cattle for expansion, but there was no difference in *S. aureus* IMI prevalence between groups that imported cattle and closed herds. However, herds that purchased replacement heifers had a greater number of *S. aureus* strains than closed herds and acquired more new strains of *S. aureus* than closed herds. Twelve of the 15 herds acquired new strains of *S. aureus* during the study. Interestingly, the three herds that did not acquire new strains of *S. aureus* were closed herds that reared their own replacements. The authors recognize that the numbers of herds within each group are small and the distribution of herds within groups is uneven,

Table. 4 *The total number of* Staphylococcus aureus *strains found within each category. Strains of* S. aureus *were identified based on the nucleic acid fragment banding patterns of* Sma*I digested DNA from individuals* S. aureus *isolates that were subjected to PFGE*

	Number of *Staphylococcus aureus* strains							
			S. aureus IMI at parturition					
Group	Herd	Heifer skin samples	Heifers	Cows	Lactating cattle	Total* strains	New† strains	Shared‡ strains
I	1	0	1	0	8	8	3	3
	2	0	0	1	11	11	3	5
	5	0	0	1	2	3	2	2
	14	6	4	0	11	13	8	7
					Mean	9	4	4
					S.E.	2	2	1
II	7	NA	0	3	5	7	3	2
	10	0	3	3	18	20	11	8
	16	9	9	3	8	17	16	4
					Mean	15‖	10¶	5
					S.E.	2	2	1
III	9	NA	0	1	2	3	1	2
	15	3	2	1	6	10	9	4
					Mean	7	5	3
					S.E.	3	3	1
IV	4	NA	0	0	0	0	0	0
	6	0	0	0	1	1	0	1
	8	0	0	0	4	4	2	3
	11	0	0	0	2	2	0	2
	12	0	2	1	3	3	2	2
	13	0	1	1	5	5	3	0
					Mean	3‖	1¶	1
					S.E.	2	2	1

* Total number of strains is not the sum of columns 2–5 as strains may have appeared in more than one category.
† Number of new strains represents the number of new strains that occurred in a herd following the initial whole herd sampling (sample period 1).
‡ Number of strains shared by at least one other herd. There was no significant difference between groups with regard to shared strains (one-way ANOVA, $P = 0{\cdot}10$).
‖ One-way ANOVA showed that group II herds had significantly more strains of *S. aureus* than group IV herds ($P = 0{\cdot}01$).
¶ One-way ANOVA showed that group II herds had significantly more new strains of *S. aureus* than group IV herds ($P = 0{\cdot}04$).
S.E., Standard error of the mean.

and therefore the results of this study should be interpreted with caution. However, the data were analysed from several different perspectives (overall prevalence, number of strains, number of new strains) and each time the results for herds that purchased replacement heifers were consistently different from one or more of the other groups studied. Previous studies have shown that replacement cattle may be a risk factor for mastitis, but no study was found that evaluated the effect of cattle importation practices on the potential for acquisition of new strains of *S. aureus* that cause mastitis. Fenlon and co-workers [13] reported that high bulk milk somatic cell count herds tended to buy replacement cattle rather than breed their own. Peeler and co-workers [14] reported that the incidence of clinical mastitis increased when the cattle replacement rate was greater than 50%. Wilson [3] presented four case examples in which herds that purchased cattle for expansion or replacement subsequently suffered a rise in bulk milk somatic cell count or acquired a contagious mastitis pathogen. Financial losses in the herds described by Wilson [3] ranged from $12308 to bankruptcy. Other studies have also shown that new strains of *S. aureus* can emerge in a

herd [6, 15], but in those reports only a single closed herd was evaluated.

Based on PFGE strain-typing we found that 82% of the *S. aureus* strain-types were unique to one herd with 12% of the strains occurring in two herds, 1% in three herds and 5% occurring in greater than three herds. These findings are similar to those reported by Joo and co-workers [10] who found that 66% of Korean *S. aureus* mastitis isolates occurred in a single herd, 27% of strains occurred in two herds, 8% occurred in three herds, and no strain occurred in greater than three herds. Others have reported that strains of *S. aureus* that cause bovine mastitis can be distributed among multiple herds [16–19]. In the present study we found that 87% of herds had a *S. aureus* strain that was shared by at least one other herd. Roberson and co-workers [20] showed that *S. aureus* strains from heifer body sites were associated with strains of *S. aureus* isolated from mammary secretions at parturition in 39% of heifers studied and that 70% of heifers with *S. aureus* IMI at parturition were infected with a strain found in lactating cow milk. In the present study we showed that *S. aureus* strains found on heifer teat and udder skin pre-partum were the same as those found in the mammary secretions of heifers at parturition 47% of the time and strains of *S. aureus* found in the mammary secretions of heifers at parturition were the same as those isolated from lactating cow milk 71% of the time. The data of Roberson and co-workers [20] and those presented here would suggest that the majority of *S. aureus* strains that cause mastitis are circulated between heifer body sites and the lactating mammary gland.

The potential for a newly acquired strain of *S. aureus* to cause an outbreak of mastitis was demonstrated by Smith and co-workers [6]. In that study an outbreak of *S. aureus* mastitis occurred following the emergence of a new strain of *S. aureus* (Novel strain) that was highly transmissible and resisted routine contagious mastitis pathogen control procedures as outlined by Neave and co-workers [5]. The outbreak of mastitis was successfully abated only after the implementation of mastitis control procedures beyond those normally considered adequate for the control of contagious mastitis pathogens [21]. Furthermore, Middleton and Fox (22) demonstrated that an IMI with Novel strain *S. aureus* resulted in significant decrease in milk production in the infected mammary quarter relative to the uninfected control quarter on the same cow ($P < 0{\cdot}01$), whereas mammary quarters infected with non-Novel strains of *S. aureus* produced similar quantities of milk to control quarters. Hence, it appears that a newly emergent strain of *S. aureus* may have the potential to be more pathogenic. Therefore, purchasing replacement heifers may put a herd at risk for an outbreak of *S. aureus* mastitis with a highly pathogenic strain.

Zadoks and co-workers [12] suggested that molecular typing techniques similar to the one described herein may be useful in identifying unique and potentially more virulent strains of *S. aureus* in order to better focus our management strategies for *S. aureus* mastitis. In the present study we demonstrate the use of PFGE as a tool for evaluating the number of strains of *S. aureus* found in a dairy herd and its potential for determining when new strains are introduced. Based on the findings of this study it seems prudent that dairy herds should, at minimum, screen purchased replacement heifers for the presence of contagious mastitis pathogens prior to co-mingling purchased replacements with resident cattle. The NAHMS 1996 dairy survey showed that only 6% of producers quarantined new animals, 9% performed individual cow milk cultures and 25% used individual cow somatic cell counts to screen for mastitis [1]. Previous studies show that new potentially more virulent mastitis pathogens that enter a dairy herd have the potential to cause an outbreak that could be financially devastating [3, 6]. Hence, to maximize herd biosecurity it appears that cattle replacement practices need to be targeted as a critical control point.

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REFERENCES

1. National Animal Health Monitoring System Dairy '96 Part 1: Reference of 1996 dairy management practices. USDA:APHIS:VS, Centers for Epidemiology & Animal Health, Fort Collins, CO. 1996: 37–40.

2. Hogan JS, Smith KL. Occurrence of clinical and subclinical environmental streptococcal mastitis. In: The Udder Health Management for Environmental Streptococci Symposium Proceedings. Ontario, 1997: 36–41.
3. Wilson D. Mastitis biosecurity: Lessons from expansion in New York. In: Proceedings of 1999 Regional Meeting of the National Mastitis Council. Waterloo: National Mastitis Council, 1999: 10–7.
4. Roberson JR, Fox LK, Hancock DD, Gay CC, Besser TE. Coagulase positive *Staphylococcus* intramammary infections in primiparous dairy cows. J Dairy Sci 1994; **77**: 958–69.
5. Neave FK, Dodd FH, Kingwill RG, Westgarth DR. Control of mastitis in the dairy herd by hygiene and management. J Dairy Sci 1969; **52**: 696–707.
6. Smith TH, Fox LK, Middleton JR. Outbreak of mastitis caused by one strain of *Staphylococcus aureus* in a closed dairy herd. J Am Vet Med Assoc 1998; **212**: 553–6.
7. Harmon RJ, Eberhart RJ, Jasper DE, Langlois BE, Wilson RA. Microbiological procedures for the diagnosis of bovine udder infection. In: Harmon RJ, Eberhart RJ, Jasper DE, Langlois BE, Wilson RA, eds. Microbiological procedures for the diagnosis of bovine udder infection, 3rd edn. Arlington: National Mastitis Council, 1990: 1–34.
8. Fox LK, Gaskins CT, Hancock DD, Newkirk D, Hutton CT. Comparison of media to isolate *Staphylococcus aureus* from teat skin and milking unit liners. Cornell Vet 1992; **82**: 225–31.
9. Devriese LA. Baird-Parker medium supplemented with acriflavin, polymixins, and sulphonamides for selective isolation of *Staphylococcus aureus* from heavily contaminated materials. J Appl Bacteriol 1981; **50**: 351–7.
10. Joo YS, Fox LK, Davis WC, Bohach GA, Park YH. *Staphylococcus aureus* associated with mammary glands of cows: Genotyping to distinguish different strains among herds. Vet Microbiol 2001; **80**: 131–8.
11. Tsen HY, Yu GK, Hu HH. Comparison of type A enterotoxigenic *Staphylococcus aureus* strains isolated from geographically far distant locations by pulsed field gel electrophoresis. J Appl Microbiol 1997; **82**: 485–93.
12. Zadoks R, van Leeuwen W, Barkema H, et al. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J Clin Microbiol 2000; **38**: 1931–9.
13. Fenlon DR, Logue DN, Gunn J, Wilson J. A study of mastitis bacteria and herd management practices to identify their relationship to high somatic cell counts in bulk tank milk. Br Vet J 1995; **151**: 17–25.
14. Peeler EJ, Green MJ, Fitzpatrick JL, Morgan KL, Green LE. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. J Dairy Sci 2000; **83**: 2464–72.
15. Davidson I. Observations on the pathogenic staphylococci in a dairy herd during a period of six years. Res Vet Sci 1961; **2**: 22–40.
16. Matthews KR, Kumar SJ, O'Connor SA, et al. Genomic fingerprints of *Staphylococcus aureus* of bovine origin by polymerase chain reaction-based DNA fingerprinting. Epidemiol Infect 1994; **112**: 177–86.
17. Fitzgerald JR, Meaney WJ, Hartigan PJ, Smyth CJ, Kapur V. Fine-structure molecular epidemiological analysis of *Staphylococcus aureus* recovered from cows. Epidemiol Infect 1997; **119**: 261–9.
18. Aarestrup FM, Wegener HC, Jensen NE, et al. A study of phage- and ribotype patterns of *Staphylococcus aureus* isolated from bovine mastitis in the Nordic countries. Acta Vet Scand 1997; **38**: 242–52.
19. Su C, Kanevsky I, Jayarao BM, Sordillo LM. Phylogenetic relationships of *Staphylococcus aureus* from bovine mastitis based on coagulase gene polymorphism. Vet Microbiol 2000; **71**: 53–8.
20. Roberson JR, Fox LK, Hancock DD, Gay JM, Besser TE. Sources of intramammary infections from *Staphylococcus aureus* in dairy heifers at first parturition. J Dairy Sci 1998; **81**: 687–93.
21. Middleton JR, Fox LK, Smith TH. Management strategies to decrease the prevalence of mastitis caused by one strain of *Staphylococcus aureus* in a dairy herd. J Am Vet Med Assoc 2001; **218**: 1615–8.
22. Middleton JR, Fox LK. Influence of *Staphylococcus aureus* strain on mammary quarter milk production. Vet Rec 2002; **150**: 411–2.