Research Article

Risk factors of *S. aureus* intramammary infection in pre partum dairy heifers under grazing conditions and molecular characterization of isolates from heifers and cows

Cecilia M. Camussone[1,2], Ana I. Molineri[1,2], Marcelo L. Signorini[1,2], Verónica E. Neder[2], Carlos A. Vitulich[2] and Luis F. Calvinho[2]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina and [2]EEA Rafaela, INTA, Santa Fe, Argentina

Abstract

The aims of the research reported here were to identify potential risk factors associated with the presence of *Staphylococcus aureus* intramammary infection (IMI) in pre partum dairy heifers on 17 dairy farms from three provinces of Argentina and to characterize, at molecular level, isolates from those heifers and lactating cows from two selected herds. A total of 1474 heifers and 4878 lactating cows were studied. The prevalence of *Staphylococcus aureus* IMI in the heifers, heifers at quarter level and lactating cow mammary quarters was 14.41, 4.82, and 14.65%, respectively. Univariate analysis showed the key variables associated with *S. aureus* IMI presence in the heifers were: *S. aureus* IMI prevalence in cows of the lactating herd, the time calves stayed with their dam after birth, the calf rearing system, the place of rearing (own farm or other dairy farm) and fly control on the farm. None of the variables included in the multivariable analysis was associated with the presence of *S. aureus* IMI in the pre partum heifers, probably due to low variability among management practices used by the farms for rearing the heifer calves. At the molecular level, *S. aureus* isolates were grouped into three main PFGE clusters and several genotypes within the clusters. Isolates from mammary secretion of pre partum heifers and milk of lactating cows comprised different PFGE clusters in both herds, although two exceptions occurred. The absence of gene *fnbpB*, which codifies for a virulence factor protein involved in cell invasion by *S. aureus*, was significantly more frequent in pre partum heifer secretion isolates than in isolates from lactating cow milk. These results suggest that, under these management conditions, isolates from mammary secretions of pre partum heifers do not originate from the milk of lactating cows, but rather other sources to which the heifer is exposed.

Worldwide, *Staphylococcus aureus* is one of the most prevalent mastitis pathogens (Persson *et al.*, 2011). In Argentina, it is a frequent cause of intramammary infection (IMI) in lactating cows (Dieser *et al.*, 2014) and has also been detected in mammary secretions of periparturient dairy heifers (Calvinho *et al.*, 2007). The prevalence of *S. aureus* IMI in pre partum heifers varied in different countries in relation to season, herd location and trimester of pregnancy (Fox, 2009). Infected mammary glands are a main reservoir of *S. aureus* since transmission of this pathogen between lactating cows is considered to occur mainly during milking time (Zecconi *et al.*, 2006). However, because infection of pre partum heifers occurs before any milking, other potential sources of *S. aureus* have been examined. Milk from lactating cows and heifer extramammary body sites (skin, muzzle) was identified as the main potential sources of IMI in pre partum dairy heifers (Roberson *et al.*, 1998). Practices for rearing heifer calves, management of heifers from first breeding to parturition, stage of pregnancy, season and herd location have been identified as risk factors for acquiring IMI before the first calving (Oliver *et al.*, 2005; Fox, 2009). Production systems and management practices vary widely, therefore, some rearing systems may have a different impact on risk factors for acquiring an IMI (Fox *et al.*, 1995; De Vliegher *et al.*, 2012). These include feeding milk from mastitis cows to calves, contact between calves, contact with adult cows, inadequate milking practices and poor housing conditions (Oliver *et al.*, 2005). Scant information is available about potential risk factors for *S. aureus* IMI in pre partum heifers in Argentina (Calvinho *et al.*, 2007).

Management practices for prevention of IMI in pre partum dairy heifers aim to decrease the risk of infection and to increase immune competence against *S. aureus* (De Vliegher *et al.*, 2012). However, detecting management practices particularly associated with prevalence of *S. aureus* IMI are a pre-requisite of applying any management practices. Any relatedness

between *S. aureus* in milk of lactating cows and from mammary secretion of pre partum heifers (Haveri *et al.*, 2008; Castelani *et al.*, 2013) or primigravid heifer colostrum (Anderson *et al.*, 2012) is unclear, specially whether or not these strains are transmitted between heifers and lactating cows within a herd.

The objectives of this study were to identify potential risk factors associated with the presence of *S. aureus* IMI in pre partum dairy heifers in dairy farms from different dairy areas of Argentina and to evaluate genetic relationships between *S. aureus* isolates from pre partum heifers and lactating cows.

Materials and methods

Herd information

Seventeen dairy herds from the Santa Fe, Córdoba and Buenos Aires provinces of Argentina were studied. Herds were selected based on (1) recognition by the dairy farmer and veterinary advisor of mastitis problems within the lactating herd, (2) willingness to participate in the study and (3) availability of animal restraint facilities to allow sampling of pre partum heifers. Herd sizes ranged from 112 to 1207 lactating cows (median 180) and average milk production of 19.2 L/cow/day. Cows were Holstein–Friesian breed. In all herds heifers were grazed mainly on alfalfa with a new area of pasture offered daily and small amounts (1 to 2 kg of DM) of hay and pasture silage fed on the grazing area. Some herds used concentrates to complement the diet; corn, sorghum grain and a commercial balanced feed with 13% crude protein were the most frequently used. Water was available ad libitum.

Survey/risk factors

A survey of management practices of heifer calves in each herd was carried out on the first visit to each farm (online Supplementary File). Risk factors explored were: *S. aureus* prevalence in lactating cows, time the calf stayed with the dam after birth, time between birth and first milking, location of calves rearing (on or off farm), calves rearing system, type of calf feeding, time calves were fed milk, rearing post-weaning (on or off farm), contact with other animals, fly control, weight at first service, clinical mastitis percentage in first third of lactation in heifers, origin of replacements.

Sampling

Composite milk samples were obtained from all lactating cows of each herd before milking using an aseptic procedure rubbing teat ends with individual cotton buds moistened with 70% alcohol prior to sampling (Oliver *et al.*, 2004). Sampling was performed the first time the farms were visited, except for one farm that had five dairy herds with one common facility for rearing heifer calves. In that case, these herds were sampled every month within a period of five months. Although culture of composite milk samples is less sensitive than quarter milk samples (Reyher and Dohoo, 2011), this procedure was selected due to the large number of animals included in the study.

Mammary gland secretion samples were collected from individual quarters of heifers approximately 20 d from the expected calving date. Heifers were immobilized in a chute, secretion samples were taken using the same aseptic procedure, after sampling the teats were dipped in a 0.5% iodophor solution. Any gross abnormality of heifers' mammary glands or teats at sampling time was recorded.

All milk and mammary secretion samples were immediately refrigerated, transported to the laboratory and cultured within 24 h.

All procedures used in this study were consistent with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 2010) and approved by the Ethics and Security Committee of the Rafaela Experiment Station from the National Institute of Agricultural Technology.

Bacteriological examination

Intramammary infection was defined based on bacteriological analysis. Milk and mammary secretion samples were cultured for mastitis pathogens according to standard procedures (Oliver *et al.*, 2004). Colony morphology, Gram staining, catalase and coagulase tests were performed. Coagulase-positive *Staphylococci* were presumptively identified as *S. aureus* and subjected to Multiplex polymerase chain reaction (PCR) for *S. aureus* identification, as previously described (Martineau *et al.*, 1998).

Molecular characterization of isolates

Two herds were selected for further characterization of *S. aureus* isolates at the molecular level. Selection criteria for the herds were a high *S. aureus* prevalence in lactating cows, different *S. aureus* prevalence in pre-calved heifers between herds, and different geographical location (online Supplementary Table S1).

Clonality of *S. aureus* isolates was assessed by Pulse field gel electrophoresis (PFGE) of *Sma*I-digested (Invitrogen™, Thermo Fisher Scientific) chromosomal DNA fragments using a CHEF-DR II apparatus (BioRad Laboratories, CA, USA) (Barbagelata *et al.*, 2012). Cluster similarity was set at 80%. Within a cluster, isolates with identical PFGE band patterns were considered to have indistinguishable genotypes (Anderson *et al.*, 2012). For within herd analysis, different clusters were identified with lower cases, and genotypes were identified with lower cases plus a numeric suffix, if needed. For between herds analysis, different clusters were identified with capital letters.

Genomic DNA was extracted for virulence gene amplification with a commercial Kit (Wizard® Genomic DNA Purification Kit, Promega) and quantified using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). The presence of *cap5* and *cap8* loci, genes for α-toxin (*hla*), β-toxin (*hlb*), Clumping factors A (*clfA*) and B (*clfB*), Fibronectin Binding Proteins A (*fnbpA*) and B (*fnbpB*), and genes from the intercellular adhesion locus, *icaA* and *icaD*, was evaluated by PCR using specific primers (online Supplementary Table 2).

Statistical analysis

The number of heifers with *S. aureus* IMI over the total number of heifers sampled within a herd at pre-calving was consider as an outcome variable. Analysis was performed in two stages: first, a univariate analysis was performed (a generalized linear model (GLM) using binomial distribution and logarithmic distribution as link function was applied). Among independent variables, for statistical purposes, *S. aureus* IMI prevalence in lactating cows was transformed into a dichotomous variable using the mean as cut-off point (>15%: high prevalence dairy farm, and <15%: low

prevalence dairy farms). In a second stage, a multivariable analysis was performed including those independent variables having a *P*-value ≤0.15 in the earlier univariate analysis (Dohoo *et al.*, 1997). Only the explanatory variable with the lowest *P*-value was selected for the multivariate model when two of them might have explained similar results and were statistically associated (collinearity evaluation).

Multivariable logistic regression using GLM was performed to evaluate the effect of the selected independent variables on the outcome variable using binomial distribution and logarithmic distribution as link function. A manually conducted backward elimination strategy was followed by removing one variable at a time with the highest *P*-value. With each variable removed from the model, the coefficient of significant variables was checked and if resulted in >20% change in estimates; the variable was retained in the model to account for its confounding effect (Chowdhury *et al.*, 2012).

For molecular characterization of *S. aureus* isolates, the associations between origin of isolate (cow *vs.* heifer) and cluster for isolates from bovine milk/secretion were examined. The association between origin of isolate (cow *vs.* heifer) and presence of a given virulence factor was examined. Statistical significance of these associations was tested by Chi-square or two-sided Fisher's exact test. All statistical analyses were carried out using InfoStat software (Universidad Nacional de Córdoba, Argentina).

Detailed materials and methods are given in the online Supplementary File.

Results

Descriptive statistics

Dairy farms that participated in the study had a total of 4878 lactating cows, with a median of 180 milking cows per herd (range 112 to 1207 cows). The prevalence of *Staphylococcus aureus* IMI in the lactating cows was 14.65% (SD = 11.08%, range 0–40%). A total of 1474 heifers were sampled from those dairy farms, with a median of 57 heifers per farm (range from 31–314). *Staphylococcus aureus* IMI prevalence in heifers at animal level was 14.41% (SD = 11.69%, range 0–41%). A total of 5896 pre-calved heifer mammary quarters were sampled, with a *S. aureus* prevalence at quarter level of 4.82% (SD = 4.02%, range 0–14.19%) (online Supplementary Table S3). Approximately 3% of the total samples (325/10 774) were contaminated.

The calves stayed with their dams >24 h after birth in 12/17 of the surveyed dairy farms. Also, in most of the farms (12/17) the first milking after calving was carried out within 24 h. The most popular calf rearing system had calves tethered by a chain to a stake (stake system), on 15 of the 17 farms. Only two of the farms reared their calves off farm in collective facilities where 4 to 6 calves were kept in small pens. On 11 of 17 farms, the calves were fed with milk or milk replacer for approximately 60 d, on two for 30 to 45 d, and on the rest of the farms for 80 d. On half of the farms (8/17) calves were fed milk from the hospital herd, on two farms milk replacer or pasteurized milk, and on the other seven farms the calves were fed milk from healthy cows. Only 3 of the 17 of the farms applied some kind of fly control.

Univariate analysis

Variables associated with *S. aureus* IMI in heifers were: *S. aureus* IMI prevalence in cows of the lactating herd (OR = 1.72 95% CI

Table 1. Final multivariable generalized lineal model (backward selection) for risk factors associated with presence of *S. aureus* in pre partum heifers

Fixed effects	Level	*P*-Value	Exp. (β)	95% CI
Intercept		**<0.001**	0.11	0.07–0.17
S. aureus prevalence in cows	<0.15 (ref.)	0.366		
	>0.15		1.24	0.78–1.97
Time the calf stayed with their dam after birth	<24 h	0.087	1.63	0.93–2.87
	>24 h (ref.)			
Calves rearing system	Stake (ref.)	0.160		
	Collective		1.38	0.88–2.17
	No (ref.)	0.084		
Fly control	Si		1.61	0.94–2.77

Bold numbers represent significant association.
Ref, reference category; CI, confidence interval.

1.24–2.39; *P* = 0.001), time calves stayed with their dam after birth (OR = 1.73 95% CI 1.19–2.51; *P* = 0.004), calf rearing system (OR = 1.72 95% CI 1.11–2.64; *P* = 0.014), place of rearing (own farm or other dairy farm) (OR = 1.82 95% CI 1.18–2.81; *P* = 0.007), and fly control on the farm (OR = 1.84 95% CI 1.15–2.93; *P* = 0.011) (online Supplementary Table 4).

Heifers from high prevalence dairy farms had 1.72 times more probability of having an *S. aureus* IMI than heifers from low prevalence dairy farms. In those herds where newborn calves stayed >24 h with their dam, the heifers had 1.73 more probability of having an *S. aureus* IMI than those herds where calves stayed <24 h. Heifers reared in collective rearing systems had 1.72 more probability of having *S. aureus* IMI than heifers reared in the stake system. Also, heifers reared off farm had 1.82 more probability of having *S. aureus* IMI than those reared on farm. Dairy farms where fly control was applied had 1.84 more probability of having *S. aureus* IMI in heifers than dairy farms where no fly control was applied (online Supplementary Table S4). However, the number of farms that declared that they had implemented fly control was low (3/17).

Multivariate analysis

None of variables included in the multivariable analysis were associated with the presence of *S. aureus* IMI pre partum in heifers at a significance level of *P* < 0.05. However, two variables showed a tendency to be associated: time the calf spent with the dam after birth (OR = 1.63, 95% CI 0.94–2.87; *P* = 0.087) and fly control on farm (OR = 1.61; 95% CI 0.94–2.77; *P* = 0.084) (Table 1). Heifers from dairy farms where calves stayed >24 h with their dams after birth had 1.63 times more probability of having *S. aureus* IMI at pre-calving than those heifers from dairy farms where calves stayed less time. Heifers from farms that had a fly control system had 1.61 more probability to be positive to *S. aureus* in comparison with heifers whose farms did not implement fly control.

Characterization of *S. aureus* isolates

In H1 three PFGE clusters, a, b and c; and six genotypes were identified: a1 (3 isolates), a2 (3 isolates), a3 (3 isolates), a4 (6 isolates), b (8 isolates) and c (10 isolates) (Fig. 1). Isolates of cluster a were commoner in heifers (93.3%) than cows (6.7%) (*P* < 0.001); clusters b and c were only present in cows (100%).
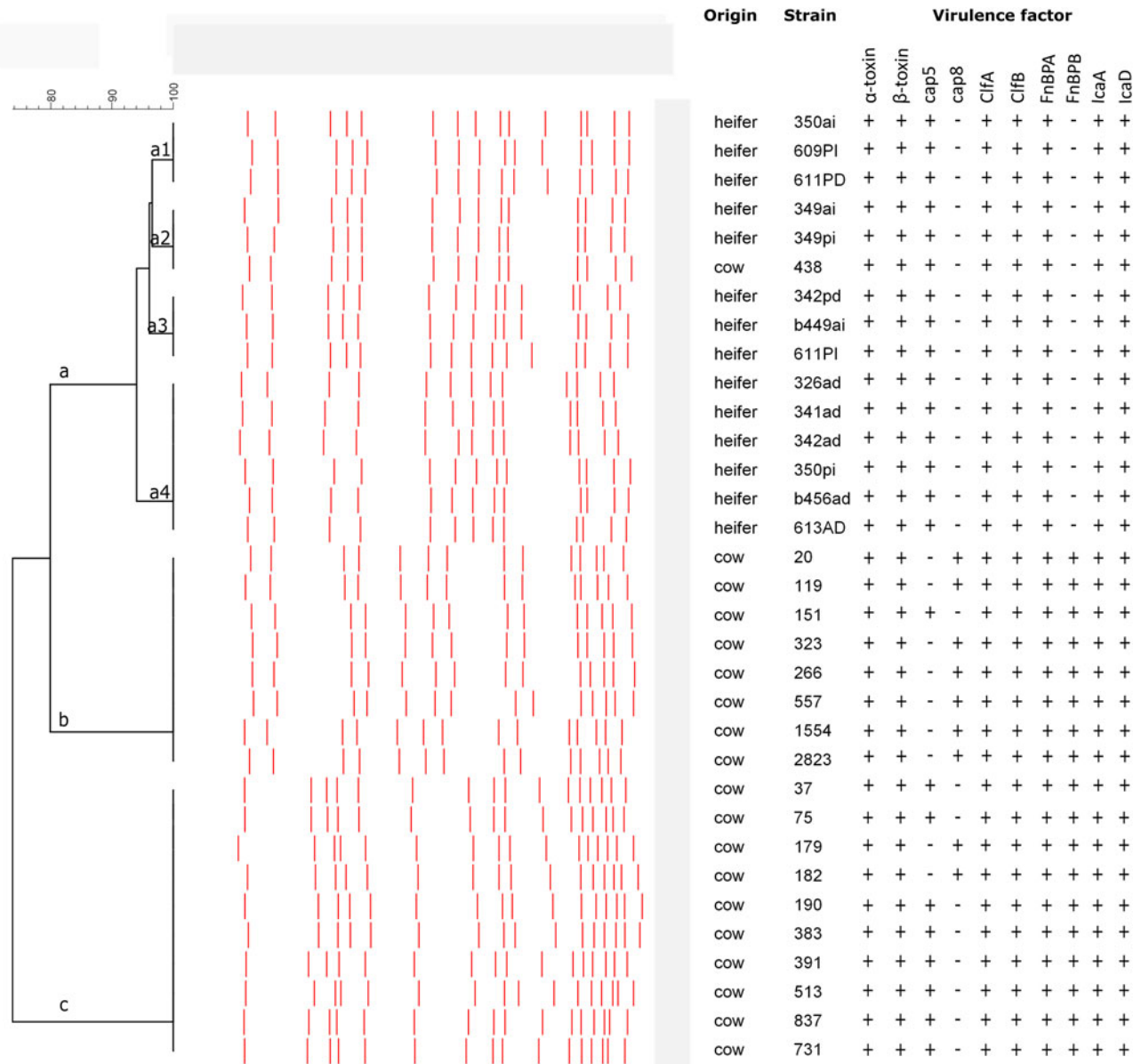
Fig. 1. Dendrogram of *S. aureus* SmaI PFGE profiles isolated from H1. Clusters were identified with lower cases (a, b and c) and genotypes were identified with lower cases plus a numeric suffix if needed. Isolates origin (cow/heifer), strain denomination, and presence (+) or absence (−) of selected *S. aureus* virulence factor genes are shown.

All *S. aureus* tested were positive for *hla*, *hlb*, *clfA*, *clfB*, *fnbpA*, *icaA* and *icaD*. All isolates in cluster a were *cap5* positive. Seven from eight isolates included in genotype b were *cap8* positive; while the remaining one was *cap5* positive. Eight from 10 isolates in genotype c were *cap5* positive, while the two remaining isolates were *cap8* positive. The sample origin (cow *vs.* heifer) was associated with the presence of *fnbpB*, being more frequent in isolates from cows (94.7%) than from heifers (0%) ($P < 0.001$).

In H2, the analysis showed two clusters, d and e, and seven genotypes: d1 (1 isolate), d2 (1 isolate), d3 (12 isolates), d4 (5 isolates), e1 (2 isolates), e2 (1 isolate) and e3 (1 isolate) (Fig. 2). Cluster e was only present in heifers ($P = 0.011$), while cluster d was commoner in cows (94.7%) than heifers (5.3%) ($P = 0.001$).

All *S. aureus* tested were positive for *cap5*, *hla*, *clfA*, *clfB*, *fnbpA*, *icaA* and *icaD*. Only the isolate included in genotype e2 was

negative for *hlb*. An association between the sample origin (cow *vs.* heifer) and presence of *fnbpB* was established, being more frequent in isolates from cows (100%) than from heifers (60%) ($P = 0.04$).

An isolate representative of each genotype identified in the analysis within herds (6 strains from H1 and 7 strains from H2) was selected (Fig. 3). No shared genotypes between herds were observed. Analysis produced thirteen individual genotypes, grouped in two clusters, A and B. Cluster A grouped eight genotypes, seven of them isolated from heifers. Genotypes a1, a2, a3, a4, e1, e2 and e3 (corresponded to pre partum heifer isolates) clustered with more than 90% of similarity, while genotype b (confirmed in lactating cow samples) was less related, clustering with 81% of similarity. Cluster B was more common in cows (80%) than heifers (20%) ($P = 0.032$). Genotypes c, d1, d3, and d4
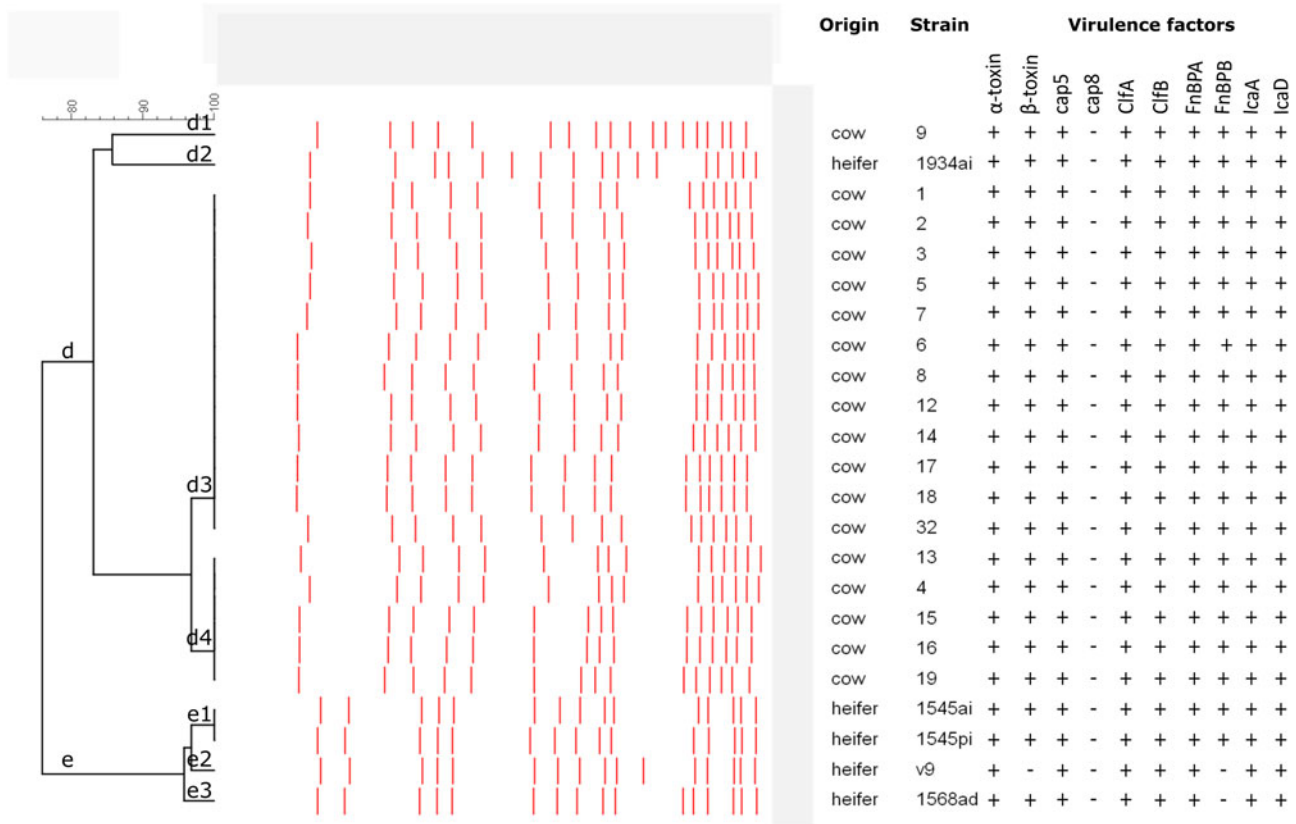
Origin	Strain	α-toxin	β-toxin	cap5	cap8	ClfA	ClfB	FnBPA	FnBPB	IcaA	IcaD
cow	9	+	+	+	−	+	+	+	+	+	+
heifer	1934ai	+	+	+	−	+	+	+	+	+	+
cow	1	+	+	+	−	+	+	+	+	+	+
cow	2	+	+	+	−	+	+	+	+	+	+
cow	3	+	+	+	−	+	+	+	+	+	+
cow	5	+	+	+	−	+	+	+	+	+	+
cow	7	+	+	+	−	+	+	+	+	+	+
cow	6	+	+	+	−	+	+	+	+	+	+
cow	8	+	+	+	−	+	+	+	+	+	+
cow	12	+	+	+	−	+	+	+	+	+	+
cow	14	+	+	+	−	+	+	+	+	+	+
cow	17	+	+	+	−	+	+	+	+	+	+
cow	18	+	+	+	−	+	+	+	+	+	+
cow	32	+	+	+	−	+	+	+	+	+	+
cow	13	+	+	+	−	+	+	+	+	+	+
cow	4	+	+	+	−	+	+	+	+	+	+
cow	15	+	+	+	−	+	+	+	+	+	+
cow	16	+	+	+	−	+	+	+	+	+	+
cow	19	+	+	+	−	+	+	+	+	+	+
heifer	1545ai	+	+	+	−	+	+	+	+	+	+
heifer	1545pi	+	+	+	−	+	+	+	+	+	+
heifer	v9	+	−	+	−	+	+	+	−	+	+
heifer	1568ad	+	+	+	−	+	+	+	−	+	+

Fig. 2. Dendrogram of *S. aureus* SmaI PFGE profiles isolated from H2. Clusters were identified with lower cases (d and e) and genotypes were identified with lower cases plus a numeric suffix. Isolates origin (cow/heifer), strain denomination, and presence (+) or absence (−) of selected *S. aureus* virulence factor genes are shown.

(corresponded to lactating cows isolates) and d2 (corresponded to one pre-calved heifer isolate) were grouped with 83% of similarity.

Discussion

Few previous studies have determined the prevalence of *S. aureus* IMI in primigravid heifers before parturition. Heifer and quarter *S. aureus* IMI found in the present study are lower than those found by Trinidad *et al.* (1990) in secretion samples from unbred and primigravid heifers in four Louisiana (USA) dairy herds (heifer IMI prevalence of 37.1% and quarter prevalence of 14.9%). However, they are similar to the *S. aureus* quarter IMI prevalence of 2.9% found by Fox *et al.* (1995) in unbred and primigravid heifers from 28 dairy herds in four states in USA and also to a previous survey in five dairy herds in Argentina with a prevalence of 4.1 of *S. aureus* quarter IMI in pre partum heifers (Calvinho *et al.*, 2007). Conversely, Aarestrup and Jensen (1997) isolated *S. aureus* from only two mammary gland quarters of 180 pre partum dairy heifers in 20 dairy farms in Denmark and Compton *et al.* (2007) found a *S. aureus* quarter prevalence of 0.4% in pre partum pasture-grazed dairy heifers in New Zealand.

Several variables were associated with *S. aureus* IMI in pre-calved heifers but no one variable was significant in the multivariable analysis. This could be caused by the homogeneity in the management practices used in the farms. Only those variables with a *P*-value less than 0.10 in the multivariable analysis were considered further.

The prevalence of *S. aureus* IMI in pre partum heifers was higher on those dairy farms where calves stayed with their dam >24 h after birth, compared with those staying <24 h. The most obvious reason is the possibility of increased transmission of *S. aureus* from the dam to the calf, which would be most likely in herds with high level of *S. aureus* IMI. This implies colonization of extramammary sites in calves. The presence of *S. aureus* on different body sites has been detected in heifer calves from birth to less than two months of age in herds with a high prevalence of *S. aureus* IMI (Matos *et al.*, 1991; Capurro *et al.*, 2010; Anderson *et al.*, 2012). Similar *S. aureus* genotypes were found both at extramammary sites of heifer calves and in the milk of lactating cows, indicating that extramammary sites are a likely source of this organism and thus the origin of heifer IMI (Capurro *et al.*, 2010; Anderson *et al.*, 2012). However, even though *S. aureus* IMI has been detected in breeding age heifers (Fox *et al.*, 1995), there is no information on the persistence at pre partum of IMI acquired during the first days of life to parturition. Therefore, the importance of this association cannot be validated.

The role of horn flies (*Haematobia irritans*) as a vector in the transmission of *S. aureus* to heifers was demonstrated in experimental studies (Owens *et al.*, 1998) and presence of *S. aureus* from the same genetic subtype was found in horn flies and mammary secretions from breeding age and bred heifers (Gillespie *et al.*, 1999). Anderson *et al.* (2012) isolated *S. aureus* of the same pulsotype from horn flies, heifer colostrum and milk from lactating cows. Therefore, fly control is recommended as a practice for prevention of IMI in dairy heifers (Oliver *et al.*, 2005; De Vliegher *et al.*, 2012). In the present study, the application of fly control was unexpectedly associated with an increased prevalence of *S. aureus* IMI. The justification for fly control in
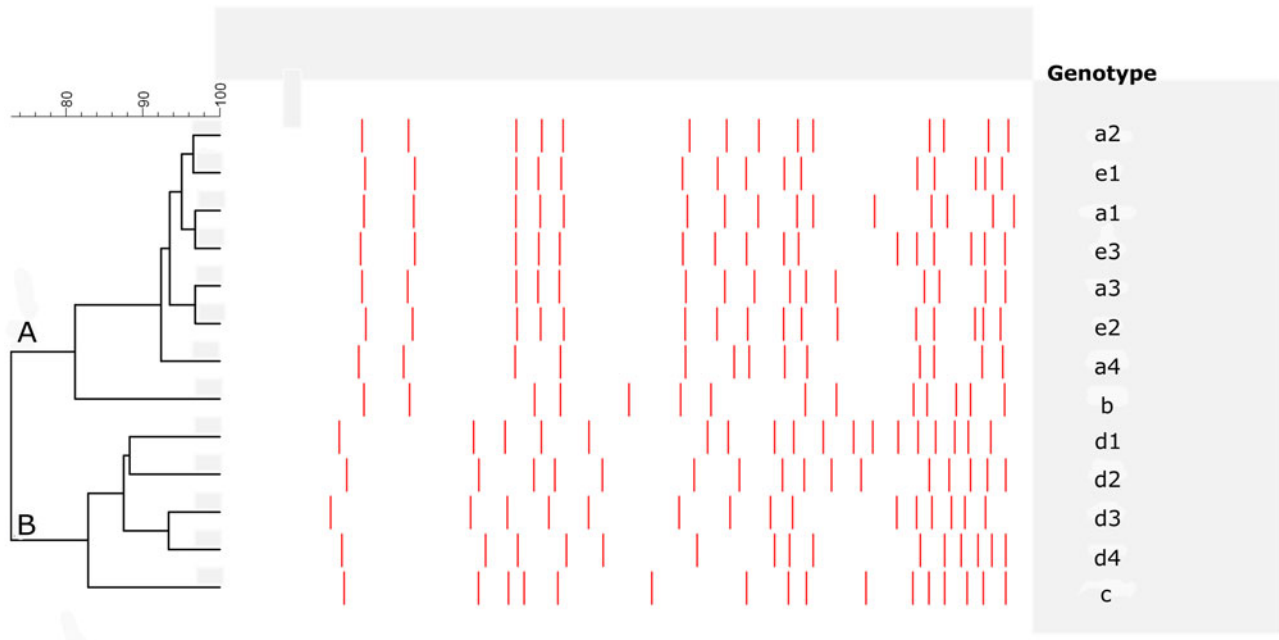
Fig. 3. Dendrogram of SmaI PFGE profiles of *S. aureus* isolates representative for each genotype defined in the analysis within herds. Clusters were identified with capital letters (A and B).

these two herds is unknown, thus the association found could be the result of cause-effect reversal, rather than a risk factor, as was observed by Nyman *et al.* (2009) for some preventive hygiene practices in herds with mastitis problems.

Heifers with *S. aureus* IMI at pre partum present a higher risk of mastitis at calving. Nearly 50% of all coagulase-positive *Staphylococcus* IMI in heifers at parturition appeared to persist into lactation for at least one month, posing a risk of transmission to uninfected herd mates (Roberson *et al.*, 1994). Previously we found that of 23 heifer quarters with a *S. aureus* IMI at pre partum, nine (39%) yielded this organism at post partum (Calvinho *et al.*, 2007). However, these studies were performed using phenotypic methods. In the present study, *S. aureus* from both IMI in lactating cows and pre partum heifers were characterized at the molecular level. Similar to previous findings (Haveri *et al.*, 2008; Capurro *et al.*, 2010; Anderson *et al.*, 2012; Castelani *et al.*, 2013), *S. aureus* isolates from the heifer herds were grouped into a few main clusters and several genotypes within some clusters. Isolates from the mammary secretion of the pre partum heifers and milk from lactating cows, in the two herds tested, grouped into different PFGE clusters in both herds, with only two exceptions. However, the time difference between when the pre partum heifers and the lactating cows were sampled does not allow drawing of any conclusions about diffusion or persistence of the *S. aureus* cluster found in heifers into the lactating herd. Our results agree with those of Castelani *et al.* (2013) who found that a *S. aureus* isolated from a pre partum heifer carried a different pulsotype from that found in lactating cows in the same herd but differ from Haveri *et al.* (2008) who found the same pulsotype in milk from lactating cows and in two heifer colostrum samples obtained at pre partum in a dairy herd with a high prevalence of *S. aureus* IMI. Anderson *et al.* (2012) found that *S. aureus* isolates from primigravid heifer colostrum and lactating cows belonged mainly to two genotypes found in both

groups. The results of the present study are not strictly comparable with those of Anderson *et al.* (2012), since in their study heifers were sampled within 3 to 4 d after calving and management practices of the newly calved heifer were not described. Our results suggest the existence of different sources of *S. aureus* infection for pre partum heifers and lactating cows, which could explain lack of statistical associations between IMI prevalence in heifers and management practices. Longitudinal analysis, including sequential sampling of heifers and lactating cows from pre-partum to the end of lactation in herds with high prevalence of *S. aureus* IMI both in pre-partum heifers and in lactating cows should be performed to provide further information about diffusion in the lactating herd of pulsotypes originated in IMI present in pre partum heifers.

All isolates carried the genes *hla*, *clfA*, *clfB*, *fnbpA*, *icaA*, *icaD* and either *cap5* or *cap8*. All isolates, except one from a heifer in H2 carried the gene for *hlb*. In H1 all isolates from cluster a and two from heifers in H2 lacked *fnbpB*. Lack of this gene was significantly more frequent in heifer isolates than in cow isolates among herds. Haveri *et al.* (2008) also detected a lack of *fnbpB* in the less common pulsotypes in two dairy herds with high prevalence of *S. aureus* IMI, but they could not establish an association between the sample origin and virulence genes. However, their study was mainly focused in the comparison of isolates from IMI and extramammary sites. In in vitro internalization assays, FnBPs were overexpressed during *S. aureus* internalization into cells, in concert with other molecules that are involved in early host-pathogen interactions (Pereyra *et al.*, 2016). Lack of *fnbpB* in heifer isolates can be associated with less internalization capacity in vivo, lower adaptation to colonize the mammary gland so resulting in a transient IMI. Conversely, presence of these genes in isolates from lactating cows can be associated with a better adaptation to colonize the mammary gland leading to a persisting IMI that could be a source of infection for other cows

in the lactating herd. Further studies including a larger number of isolates and herds with high prevalence of *S. aureus* IMI are needed to confirm the trend observed in the present study and also to detect other genotypic characteristics linked to adaptation of this organism to the mammary gland (Bardiau *et al.*, 2016).

In conclusion, none of the variables studied proved to be associated with the presence of *S. aureus* IMI in pre partum heifers according to multivariable analysis, probably due to the low variability among management practices used for rearing heifer calves, although the time calves stayed with their dam after birth and fly control on the farm showed a tendency for association. *Staphylococcus aureus* isolates from mammary secretions of pre partum heifers and milk of lactating cows were grouped in different PFGE clusters in both herds. The absence of the *fnbpB* gene was significantly more common in isolates from pre-calved heifer mammary secretion than in those of lactating cow milk among both herds studied. Altogether these results suggest that under the particular management conditions applied in Argentina isolates from mammary secretions of pre-calved heifer do not originate from the milk of lactating cows, but rather from other sources to which the heifer is exposed.

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