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Intramammary infections during the periparturient period in Argentine dairy heifers

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ABSTRACT

Prevalence of intramammary infections at prepartum and postpartum in primigravid heifers from five dairy herds located in the central dairy area of Argentina was determined. Mammary secretion samples from 140 heifers (560 mammary quarters) were obtained 14 days prior to the expected calving day and within 7 days after parturition and subjected to bacteriological analysis. No clinical mastitis cases were detected during the study. The number of infected heifers in at least one mammary quarter at pre and postpartum was 87 (62.2%) and 53 (37.8%), respectively. The most prevalent mastitis pathogens at prepartum among samples yielding a positive bacteriological culture were coagulase-negative staphylococci (69.07%), *Staphylococcus aureus* (12.71%) and *Streptococcus uberis* (4.42%). A decrease on isolation frequency of coagulase-negative staphylococci (53.41%) and *S. uberis* (2.27%) was observed at postpartum, while that of *S. aureus* showed an increase (21.59%). Presence of intramammary infections appeared to be associated with some management conditions. These results highlighted the need to improve diagnosis and control measures in replacement heifers.

Key words: heifers, mastitis, peripartum

RESUMEN

Infecciones intramamarias durante el periparto en vaquillonas en Argentina. Se determinó la prevalencia al preparto y posparto de infecciones intramamarias causadas por organismos patógenos de mastitis en vaquillonas primíparas de cinco establecimientos lecheros ubicados en la cuenca central santafesina. Se tomaron muestras de secreción mamaria de 140 vaquillonas (560 cuartos mamarios) aproximadamente 14 días antes de la fecha probable de parto y dentro de los 7 días posparto, y se procesaron bacteriológicamente. No se detectaron casos de mastitis clínicas durante el estudio. El número de vaquillonas infectadas en al menos un cuarto mamario al preparto y posparto fue de 87 (62,2%) y 53 (37,8%), respectivamente. Los organismos patógenos más prevalentes al preparto entre las muestras con cultivo bacteriológico positivo fueron estafilococos coagulasa negativos (69,07%), *Staphylococcus aureus* (12,7%) y *Streptococcus uberis* (4,42%). Al posparto se observó un descenso en la frecuencia de aislamiento de estafilococos coagulasa negativos (53,41%) y *S. uberis* (2,27%), mientras que la de *S. aureus* mostró un aumento (21,59%). La presencia de infecciones intramamarias pareció estar asociada con algunas prácticas de manejo. Estos resultados ponen de manifiesto la importancia de hacer extensivo el diagnóstico y control de la enfermedad a las vaquillonas de reemplazo antes de su ingreso al rodeo en ordeño.

Palabras clave: vaquillonas, mastitis, periparto

INTRODUCTION

Early studies showed that intramammary infections (IMI) in primiparous cows have a significant effect on milk production during subsequent lactations (9). Many of these infections are associated with elevated somatic cell counts, can persist for long periods, may impair mammary development and affect milk production after calving (6, 22). Prevalence of IMI in breeding age and pregnant heifers is variable and can be caused by different mastitis pathogens at pre and postpartum (5, 12, 18, 23). However, there is only limited information about prevalence of IMI in primigravid heifers under management conditions found in South America (3, 4). The objectives of this study were to determine the prevalence of IMI in primigravid heifers during the periparturient period and to assess the association of selected calf management practices with the presence of IMI at prepartum in heifers from five dairy herds of the central dairy area of Argentina.

MATERIALS AND METHODS

Animals

Heifers from five dairy herds located in the central dairy area of Argentina were used. Herds were selected based on their willingness to cooperate in the study, distance from the laboratory and availability of restraint facilities. Information on management practices from herds included in this study was collected by means of a structured questionnaire and from farm records (Table

1). A total of one hundred and forty primigravid Holstein heifers (Farm A: n=49, Farm B: n=9, Farm C: n=47, Farm D: n=22, and Farm E: n=13) participated in this research. In all herds heifers were reared under grazing conditions and 20 days prior to the expected calving day were located in a calving paddock together with adult cows. Heifers were sampled between 1999 and 2001.

Experimental procedures

Quarter mammary secretions were aseptically obtained in sterile plastic tubes within 14 days prior to expected parturition and 7 days after calving. A total of 560 mammary quarters were sampled before and after parturition. Prior to sample collection teat orifices were disinfected using a cotton bud impregnated with 70% alcohol according to recommended procedures (7), and an approximate volume of 3 ml was collected from each quarter. Quarters were dipped in a 0.5% iodophor solution after sample collection. Mammary secretion samples were immediately refrigerated and stored frozen at -20°C for one to two months before laboratory processing.

Bacteriological procedures

Milk samples were thawed at room temperature and 0.01ml were streaked on quarter plates of Columbia agar (Britania, Buenos Aires) supplemented with 5% ovine blood with a sterile loop. Plates were incubated at 37 °C and were examined for bacterial growth at 24 and 48 h. Isolates were identified according to standard procedures (7). Briefly, catalase-positive cocci were identified according to colonial morphology, hemolysis production, Gram stain, and coagulase tube test. Coagulase-positive staphylococci were presumptively identified as *Staphylococcus aureus.* Catalase-negative cocci were identified according to colonial morphology, hemolysis production, Gram stain, CAMP test, esculin and hippurate hydrolysis, and growth in 6.5% NaCl broth. Gram-negative bacteria were identified according to colonial morphology, Gram stain, oxidase test, reaction in triple sugar iron agar (Britania, Buenos Aires), in sulfide-indol-motility medium (Britania, Buenos Aires) and in Koser citrate medium (Britania, Buenos Aires). *Corynebacterium* spp. and *Bacillus* spp. were identified based on colony appearance on blood agar, catalase test and Gram stain. Organisms were tentatively identified as yeasts according to colony morphology, Gram stain and microscopic morphology. Mammary secretion samples yielding more than two different colony types were considered to have been contaminated during sample collection (5). Quarters were categorized as infected with minor or major pathogens according to classical definitions (8).

Interpretation and analysis of culture data

Culture data were expressed as percent of the total samples examined at prepartum and postpartum periods. Percentages in some cases did not total 100 because more than one mastitis pathogen could be isolated from the same sample. In addition, a percentage of heifers infected quarters at prepartum and postpartum was analyzed. An exploratory analysis was carried out to assess probable risk factors for IMI at prepartum. All variables were categorical and compared by means of Chi square and odds ratio. The dependent variable was IMI categorized as caused by major pathogens, minor pathogens and absence of infection. The latter was regarded as the reference group. Quarters were considered to be exposed to: calf age at weaning (≤ 60 days), time calf spent with dam after birth (≤ 1 day), and calf fed with mastitic milk before weaning (yes/no).

RESULTS

A total of 1120 foremilk samples were examined bacteriologically during this study. Bacteriological findings at pre and postpartum are shown in Tables 2 and 3. The most frequently isolated organisms both at pre and postpartum were coagulase-negative staphylococci (CNS), followed by *S. aureus* and *Streptococcus* species. Among the latter, *Streptococcus uberis* was the most frequently isolated species at prepartum. Only in one case *Streptococcus agalactiae* was detected in a prepartum sample (Farm C) in a mixed culture with *S. aureus*. Frequency of bacterial isolation among samples yielding a positive culture at pre and postpartum is shown in Tables 4 and 5. Approximately 33% of quarters were infected either by major or minor mastitis pathogens at prepartum while 15.7% of quarters were infected at postpartum. A total of 62.2% and 37.8% of heifers were infected in at least one mammary quarter at prepartum and postpartum, respectively. The distribution of infected quarters among heifers

Table 1. Summary of heifer management practices in five selected dairy herds located in the central dairy area of Argentina.

	Herds				
Practice	A Research	B Commercial	C Commercial	D Commercial	E Commercial
Time calf w/dam after birth	2-5 days	5-7 days	1 day	12 h	1 day
Feeding before weaning	Milk (mastitic and non mastitic)	Milk (mastitic and non mastitic)	Milk (mastitic and non mastitic)	Colostrum for 3 days and milk replacer	Mastitic milk and colostrum
Housing before weaning	tether	tether	tether	tether	tether
Age at weaning (days)	55	60-70	70	50-60	60
Age at first breeding (mo)	15-18	15-17	16	16-18	18

Table 2. Bacteriological findings from mammary quarter secretions of primigravid heifers prior to parturition in five selected dairy herds located in the central dairy area of Argentina.

Outcome	Farm A n (%)	Farm B n (%)	Farm C n (%)	Farm D n (%)	Farm E n (%)	Total n (%)
Positive single culture	49 (25)	15 (41.67)	69 (36.7)	17 (19.32)	13 (25)	163 (29.11)
Positive mixed culture	4 (2.04)	-	5 (2.66)	-	-	9 (1.61)
Contaminated	3 (1.53)	1 (2.78)	4 (2.13)	-	-	8 (1.43)
Negative	140 (71.43)	20 (55.55)	110 (58.51)	71 (80.68)	39 (75)	380 (67.86)
Total quarters	196	36	188	88	52	560

Table 3. Bacteriological findings from mammary quarter secretions of primigravid heifers after parturition in five selected dairy herds located in the central dairy area of Argentina.

Outcome	Farm A n (%)	Farm B n (%)	Farm C n (%)	Farm D n (%)	Farm E n (%)	Total n (%)
Positive culture	35 (17.86)	3 (8.33)	26 (13.83)	18 (20.45)	6 (11.54)	88 (15.71)
Non functional	-	1 (2.78)	-	-	-	1 (0.18)
Contaminated	-	3 (8.33)	1 (0.53)	2 (2.28)	-	6 (1.07)
Negative	161 (82.14)	29 (80.56)	161 (85.64)	68 (77.27)	46 (88.46)	465 (83.04)
Total quarters	196	36	188	88	52	560

Table 4. Distribution of bacterial species among samples yielding a positive culture from mammary secretions of primigravid heifers prior to parturition in five selected dairy herds located in the central dairy area of Argentina.

Bacteria	Farm A n (%)	Farm B n (%)	Farm C n (%)	Farm D n (%)	Farm E n (%)	Total n (%)
CNS [(1)]	43 (75.44)	14 (93.33)	52 (65.82)	10 (58.82)	6 (46.15)	125 (69.07)
Saphylococcus aureus	4 (7.02)	-	13 (16.46)	2 (11.77)	4 (30.77)	23 (12.7)
Streptococcus uberis	3 (5.26)	-	4 (5.06)	-	1 (7.7)	8 (4.42)
Other streptococci [(2)]	1 (1.75)	-	7 (8.86)	-	-	8 (4.42)
Other bacteria [(3)]	6 (10.53)	1 (6.67)	3 (3.8)	5 (29.41)	2 (15.38)	17 (9.39)
Total	57	15	79	17	13	181

[(1)]CNS: coagulase-negative staphylococci.
[(2)]*Streptococcus agalactiae*, *Streptococcus dysgalactiae,* and *Streptococcus* spp.
[(3)]*Serratia* spp., *Klebsiella pneumoniae*, *Proteus* spp., and *Bacillus* spp.

Table 5. Distribution of bacterial species among samples yielding a positive culture from mammary secretions of primigravid heifers after parturition in five selected dairy herds located in the central dairy area of Argentina.

Bacteria	Farm A n (%)	Farm B n (%)	Farm C n (%)	Farm D n (%)	Farm E n (%)	Totaln (%)
CNS [(1)]	16 (45.71)	2 (66.67)	13 (50)	14 (77.78)	2 (33.33)	47 (53.41)
Staphylococcus aureus	4 (11.43)	-	8 (30.77)	3 (16.67)	4 (66.67)	19 (21.59)
Streptococcus uberis	-	-	2 (7.69)	-	-	2 (2.27)
Other streptococci [(2)]	2 (5.72)	-	1 (3.85)	-	-	3 (3.41)
Other bacteria [(3)]	13 (37.14)	1 (33.33)	2 (7.69)	1 (5.55)	-	17 (19.32)
Total	35	3	26	18	6	88

[(1)]CNS: coagulase-negative staphylococci.
[(2)]*Streptococcus dysgalactiae* and *Streptococcus* spp.
[(3)]*Escherichia coli*, *Bacillus* spp., *Corynebacterium* spp., and yeasts.

Table 6. Frequency of mammary quarters infected at pre and postpartum in dairy heifers from selected dairy herds located in the central dairy area of Argentina.

		Mammary quarters infected							
		Prepartum				Postpartum			
Farm	n	Uninfected n (%)	One quarter	two quarters	≥ 3 quarters	Uninfected n (%)	one quarter	two quarters	≥ 3 quarters
A	49	21 (42.9)	11 (22.4)	10 (20.4)	7 (14.3)	28 (57.1)	12 (24.5)	5 (10.2)	4 (8.2)
B	9	2 (22.2)	2 (22.2)	3 (33.4)	2 (22.2)	6 (66.7)	3 (33.3)	-	-
C	47	14 (29.8)	13 (27.7)	5 (10.6)	15 (31.9)	32 (68.1)	7 (14.9)	5 (10.6)	3 (6.4)
D	22	12 (54.5)	6 (27.3)	2 (9.1)	2 (9.1)	13 (59.1)	3 (13.6)	4 (18.2)	2 (9.1)
E	13	4 (30.8)	7 (53.8)	1 (7.7)	1 (7.7)	8 (61.5)	4 (30.8)	1 (7.7)	-
Total	140	53 (37.8)	39 (27.9)	21 (15)	27 (19.3)	87 (62.2)	29 (20.7)	15 (10.7)	9 (6.4)

Table 7. Risk factors for prevalence of intramammary infection in heifers at prepartum in five selected dairy farms located in the central dairy area of Argentina.

Variable		Major		Minor		No infection	Total
		n	OR[1]	n	OR[1]	n	
Age at weaning	>60 days	15	3.6	23	1.5	19	
	<60 days	9		34		41[2]	141
Time calf w/dam	<1 day	19	2.5	28	0.6	36	141
	>1 day	5		29		24[2]	
Feeding mastitic milk	Yes	22	3.0	50	2.0	47	141
	No	2		7		13 NS[3]	

[1]OR: odds ratio; [2]p< 0.05; [3]NS: not significant.

in the different dairy farms is shown in Table 6. From 23 quarters that presented *S. aureus* IMI at prepartum, nine (39%) also yielded this organism at postpartum; while from 125 quarters infected with CNS at prepartum, seventeen (13.6%) yielded these organisms at postpartum.

Mammary quarters exposed to calf age at weaning > 60 days, and time calf spent with dam after birth < 1 day had respectively 3.6 and 2.5 higher risk to have IMI at prepartum (p< 0.05). Although quarters from heifers fed mastitic milk also appeared to have a higher risk than those from heifers that were fed only colostrum and milk substitute, that association was not statistically significant (Table 7).

DISCUSSION

Intramammary infections in primiparous dairy heifers have been early recognized; however, preparturient infections were considered to occur rarely (12). Several studies carried out over the last two decades showed that prevalence of IMI in heifers before parturition was higher than previously expected (18). In this study, 62.3% of heifers and 32% of quarters were infected at prepartum, which is lower than other authors' findings. Trinidad *et al.* (22) carried out a survey in four dairy herds in Louisiana and found 96.6% of heifers and 74.6% of quarters infected at prepartum. In addition, these authors found that 29% of heifers and 15.1% of quarters showed clinical mastitis symptoms, while no clinical cases were observed at prepartum in the present study. In a survey that included 120 heifers in six dairy herds in São Paulo, Brazil, 80% of heifers and 53% of quarters were infected at prepartum (4). Divergence of results among studies can be related to location, which has been linked to management styles used in different areas, as was observed in a survey including 28 dairy herds in four states of the USA (5). Prevalence of IMI in heifers at first parturition was also found to be influenced by stage of pregnancy season and herd (5). The associations found in the present study between IMI at prepartum, calf age at weaning and time that calf spent with dam after birth may only prove that a link exists between heifers´ IMI and management practices. Moreover, since the latter tend to be homogeneous within a given herd, these associations may only

reflect differences among herds due to other causes. Since only a small number of dairy farms were studied, further research is needed to point out specific risk factors and to rule out the chances of interaction or/and confounding.

Prevalence of IMI in primigravid heifers at calving has been reported to range between 26% to 45% in studies carried out on two and eleven dairy herds, respectively (11, 19); while in the present study 37.8% of heifers were infected at post partum sampling. The number of infected quarters at prepartum and postpartum sampling decreased from 33 to 16%, respectively. Coagulase-negative staphylococci were the most prevalent bacteria isolated either at pre and postpartum. This finding is in accord with previous studies that included prepartum (4, 5, 22) and postpartum samples (5, 19). The number of quarters infected with CNS decreased from prepartum to postpartum in all herds included in this study, which agrees with previous observations (5, 15) that suggest that some CNS infections are spontaneously eliminated upon milking or that some infections identified as intramammary at prepartum are actually the result of transient teat duct colonization. These organisms are either part of the normal udder skin flora or free-living in the environment and some species, like *Staphylococcus chromogenes*, are known to persist for longer periods than other CNS (10, 18). In addition, there appears to be a correlation between colonization of the teat canal by some CNS species and IMI (13) and an association between management practices and species distribution in a herd (10). However, the pathogenic significance of CNS is still uncertain.

Identification of coagulase-positive staphylococci (CPS) to species level was beyond the scope of this study. Although CPS other than *S. aureus* can be isolated from bovine milk, previous studies agreed that most CPS isolated from bovine mastitis are *S. aureus* (2, 20). *S. aureus* was found either before and after parturition in the majority of farms surveyed and was the most prevalent major pathogen identified in this research, which agrees with previous studies (5, 22). In addition, in a previous study carried out in Entre Ríos, Argentina, high frequency of isolation of *S. aureus* at prepartum was observed in heifers from a dairy herd with high prevalence of staphylococcal IMI (3). *S. aureus* is a major mastitis pathogen that causes extensive tissue damage, is refractory to antibiotic therapy and is mainly transmitted from cow to cow during milking time (21). Presence of *S. aureus* in primiparous heifers at prepartum is of great concern due to effects of infection in milk production in ensuing lactation. In addition, in the present study 39% of quarters yielding this organism at prepartum were still infected following parturition, thus becoming a potential source of infection during milking time. Considering that *S. aureus* is the most prevalent mastitis pathogen in Argentina (1), further studies will be needed to determine risk factors for infections in heifers under management conditions currently used in Argentina.

S. uberis was the most frequently isolated streptococcal species before parturition; however, only few IMI caused by this organism were detected at postpartum. Environmental streptococci were the most prevalent pathogens in studies carried out in herds with low prevalence of contagious mastitis pathogens (14, 16, 17). These findings led to hypothesize that bacteria causing IMI in pregnant heifers likely reflect prevalence of IMI in lactating cows (18). In only one case *S. agalactiae* was isolated from a prepartum sample in a mixed infection with *S. aureus*. Early studies demonstrated that feeding raw milk contaminated with *S. agalactiae* and allowing female calves to suckle each other at will was associated with shedding of this organism at parturition (21). Although detection of the origin of infections was beyond the scope of this study, feeding calves with milk from cows with mastitis is a widespread practice among dairy herds in the central dairy area.

In conclusion, results of this study corroborated that presence of IMI in heifers under common management conditions used in the central dairy area is a frequent event. Current mastitis control methods are aimed at preventing new IMI and at eliminating existent IMI either during lactation or the nonlactating period (21). Control practices based on hygiene during milking time are directed to prevent new IMI and antibiotic therapy during lactation is mainly recommended for clinical mastitis cases. Hence, these practices are not effective to control IMI in heifers entering the milking herd. These features highlight the need to determine risk factors for the most prevalent organisms causing IMI in heifers under current management conditions and to establish control practices directed to minimize the presence of infections in replacement heifers.

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