Epidemiology of Mastitis in Pasture-Grazed Peripartum Dairy Heifers and Its Effects on Productivity

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ABSTRACT

An observational field study was conducted on 708 heifers in 30 spring-calving dairy herds in the Waikato region of New Zealand. The aim of the study was to describe patterns and effects of intramammary infection (IMI) and clinical mastitis (CM) in the peripartum period. Mammary secretion samples for bacteriological testing were taken from all quarters approximately 3 wk before the planned start of the calving period and within 5 d following calving, in addition to quarters diagnosed with CM within 14 d of calving. Precalving IMI was diagnosed in 18.5% of quarters, and of these, coagulase-negative staphylococci were the predominant isolate (13.5% of quarters). *Streptococcus uberis* prevalence increased 4-fold to 10.0% of quarters on the day of calving compared with the precalving period. Prevalence of all pathogens decreased rapidly following calving. Clinical mastitis cases were predominantly associated with *Strep. uberis* (64%). The daily hazard of diagnosis was higher in heifers than in cows (0.06 vs. 0.02/d on d 1 postcalving, respectively), but was not different by d 5 (0.005 vs. 0.002, respectively) of lactation. Intramammary infection with a major pathogen was associated with an increased risk of removal from the herd (15 vs. 10% for infected and noninfected heifers, respectively) and somatic cell count >200,000 cells/mL at subsequent herd tests (15 vs. 8%), but neither CM nor IMI were associated with reduced milk yield or milk solids production. Results suggest that bacterial species involved and the pattern of IMI prevalence in pasture-grazed peripartum heifers differ from those in other production systems. Further, mastitis control programs need to target major environmental pathogens causing precalving IMI, because new infections are likely before the onset of lactation, whereas existing detection and control measures are generally implemented after calving. Novel control programs that re-

duce new infections due to *Strep. uberis* immediately before calving are required to reduce the incidence of CM in pasture-grazed dairy heifers.

Key words: mastitis, epidemiology, heifer, peripartum

INTRODUCTION

Heifers (2-yr-old primiparous cattle) have a high incidence of clinical mastitis (CM) in the peripartum period relative to older animals in herds. Studies reported a high incidence of CM and IMI in first-calving heifers immediately following calving (Pankey et al., 1991; Barkema et al., 1998; Barnouin and Chassagne, 2001). A high prevalence of IMI was reported in heifers before calving (Trinidad et al., 1990), and a positive association was found between pre- and postpartum infection (Oliver and Sordillo, 1988; Aarestrup and Jensen, 1997). Yet, the incidence and prevalence of IMI in peripartum heifers varies between locations and management systems (Myllys and Rautala, 1995; Waage et al., 1998).

Milk yield losses reported in heifers diagnosed with peripartum CM are variable, from less than 1% (Myllys and Rautala, 1995; Barnouin and Chassagne, 2001) to 5% (Oltenacu and Ekesbo, 1994) over a lactation, and 2.5 kg/d in the 7 d following *Streptococcus* spp. cases in the first week of lactation (Grohn et al., 2004).

First-calving heifers represent a valuable current and future resource. They make up the largest parity group in most herds, usually have the highest genetic merit of any age group in the herd, and, until a calf or milk is sold following their first calving, have not generated any revenues. For these reasons, diseases occurring at high frequency, and adversely affecting the production and lifetime performance of heifers must be a serious concern of dairy producers.

Little information has been published on the epidemiology of peripartum mastitis in pasture-grazed dairy heifers. Pankey et al. (1996) reported that 35% of heifers in pasture-grazed herds in New Zealand had one or more quarters diagnosed with IMI within 5 d following calving, and 8% of heifers had CM in the same period. But there are no data on the prevalence of IMI before

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calving in heifers in pasture-grazing farming systems, or on any productivity effects following naturally occurring IMI pre- or postcalving, or following CM in these systems.

Hence, the main aims of this study were to 1) describe at the quarter and heifer level the prevalence of IMI several weeks before, and within 5 d following calving, and 2) describe the incidence of CM in the first 14 d of lactation in first-calving heifers in pasture-grazed dairy herds. The study aimed to describe the bacteria involved in heifer peripartum IMI and CM and the repeatability of bacterial isolations over time. Additional aims were to estimate the risk of thelitis and loss of quarter symmetry or function by mid lactation, individual SCC (ISCC), milk yield and milk solids production at first postpartum production recording and averaged over the lactation, and the risk of premature culling in heifers.

MATERIALS AND METHODS

Herd and Heifer Selection

Thirty spring-calving dairy herds were selected for a prospective observational study within a 30-km radius of Morrinsville in the Waikato region of New Zealand. The selected herds routinely used DHI production recording 4 times during lactation, used an electronic database for the recording of individual animal details including breed, date of calving, and milk production records, and were willing to follow the study protocol. Before animal enrollment, informed consent to participate was gained from each herd owner and approval for the study to proceed was given by an animal ethics committee. Average herd size was 332 ± 138 (standard deviation) cows and the average planned start of the seasonal calving period was July 13, 2004 (± 7 d). The average milk yield and milk solids (milk fat and milk protein) production for all cows was $3,843 \pm 709$ kg and 331 ± 53 kg, respectively. For first-parity heifers, production was $3,190 \pm 648$ kg and 279 ± 43 kg, respectively. A systematic random selection of heifers in each herd that were due to calve in the season of the study were enrolled on one calendar date, approximately 3 wk before the planned starting date of the seasonal calving period. This meant that the interval between enrollment and calving for individual heifers varied between 3 wk and >10 wk. A total of 708 heifers were enrolled in this study. Twenty of the 30 herds each contributed 27 heifers, and the other 10 herds each contributed between 6 and 26 heifers. Heifer breeds were Friesian ($n = 291$), Jersey ($n = 214$), and other (predominantly Friesian-Jersey crossbreed, $n = 203$). Heifers diagnosed with CM by the farmers were treated with i.m. antibiotics with label claims for treatment of IMI. Enrolled heifers were managed with the others of

the same parity group in a consistent way until calving for all heifers was completed. Diet was predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) fed in situ, with a new area of pasture offered daily and with small amounts (1 to 2 kg of DM) of hay and pasture silage fed on the grazing area. Data on CM (treatment date, cow identity) and calving dates were available from all cows in the study herds.

Sample and Data Collection

At the time of enrollment, a single sample of mammary secretion was collected from each gland for bacteriologic examination following aseptic preparation of the teat end (only a single sample could be taken because of the low volume of secretion available). A commercial iodine-based teat antiseptic with 0.5% available iodine was applied by spray to the teats immediately after sampling. Duplicate milk samples were collected using the same method from all glands of each heifer within 5 d of calving during preplanned twice-weekly visits to the herds by trained technicians. If CM was diagnosed before a planned visit, duplicate milk samples were taken from all glands by a trained technician. Duplicate milk samples were collected from all first cases of CM occurring after the preplanned 1 to 5 d period; that is, between 6 and 14 d of lactation. On each sampling occasion, any abnormalities of the glands or teats were recorded. Heifer data including breed, New Zealand EBV, calving date, and individual animal milk production records were obtained electronically from a database (Livestock Improvement Corporation, Hamilton, New Zealand). All individual animal disease treatments from 1 mo before enrollment date and reason for removal of any cows from the herds throughout the lactation were collected from farm records. Results of microbiological tests of milk from heifers treated with systemic or intramammary antibiotics in the 21 d preceding sampling were excluded from analysis. At approximately 3 mo after the start of the calving period, each heifer was examined for the presence of thelitis (defined as a manually detectable thickening of the teat canal) and for the presence of nonfunctional or scantily functional mammary glands (defined as a visually apparent smaller gland compared with the contralateral gland in the same heifer immediately before attachment of milking units).

Bacteriological Examination

Microbiological procedures, diagnosis of IMI, and categorization of results were undertaken using standard methodology (Hogan et al., 1999). Milk samples were mixed thoroughly by inverting 2 to 3 times and then

10 μ L was streaked onto a quarter plate of 5% sheep blood, 0.1% esculin agar (Fort Richard, Auckland, New Zealand) using a sterile disposable loop. Plates were incubated at 37°C for 48 h before reading results. All gram-positive, catalase-negative cocci were categorized as streptococci and further speciated by their esculin reaction, then CAMP (Christie, Atkins, Munch-Peterson) test results. Gram-positive, catalase-positive cocci were coagulase tested using a commercial kit (BBL Staphyloslide Latex test, Becton Dickinson, Sparks, MD) and categorized as either CNS or coagulase-positive (assumed to be *Staphylococcus aureus*). Gram-negative rods that could be identified with the basic biochemical tests (lactose, oxidase, triple sugar iron, Simmons citrate, and motility) were identified and recorded, and unidentified organisms were recorded as gram-negative rods. Gram-positive rods that could be identified with simple procedures were identified and recorded (e.g., *Corynebacterium* spp.). *Bacillus* organisms were identified by morphology only and recorded as *Bacillus* spp.; any unidentified organisms in this group were recorded as gram-positive rods. The number of colonies of each colony type was counted, up to a maximum of 50. Samples with more than 2 colony types were defined as contaminated. Samples from which fewer than 3 colonies of any 1 type of organism were found were recorded as a no growth, except for *Staph. aureus* where ≥ 1 colony was recorded as an isolate. When duplicate samples were collected, both samples were required to have >2 colonies of the same bacterial species for the glands to be defined as infected. If 1 of the duplicate samples was contaminated, the results from the uncontaminated duplicate alone were used to diagnose infection.

Data Handling

Bacterial isolates were categorized as either major or minor pathogens. Bacterial species classified as major pathogens were *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Staph. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, and *Strep. uberis*. Minor pathogens were CNS, *Corynebacterium* spp., undifferentiated gram-negative rods, undifferentiated gram-positive rods, and yeasts. Bacteriological results from cases of CM that occurred within 0 to 5 d following calving were defined as IMI. Thus, the quarters defined as having an IMI also include those that were diagnosed with CM (this occurred in 195 quarters in 163 heifers). Clinical mastitis quarters were evaluated separately. Where a second pathogen was isolated from the gland, it was recorded as "isolate 2." Results of bacteriological testing of milk samples on one occasion and for repeated sampling

were summarized at the quarter-level according to the definitions in Table 1. When a quarter had both a major and a minor pathogen isolated at the same time, the quarter was given major pathogen status for that sampling occasion ($n = 46$ quarters precalving and 55 quarters postcalving), and the major pathogen was reported. When describing bacteriological results from samples taken at different occasions, the individual quarter bacterial isolates were compared. More than 1 bacterial isolate was identified from some quarters, and therefore, the categories for bacteriological status between samplings for a quarter were not mutually exclusive. For example, a quarter could be classified as both IMI "same" and IMI "new" if 1 of the precalving bacterial isolates was present at both pre- and postcalving samples (e.g., *Staph. aureus*) and a new bacteria was isolated postcalving (e.g., *E. coli*). Results of bacteriological testing of milk samples were aggregated from quarter to heifer level using the same major or minor pathogen quarter definitions. Results of heifer-level data were aggregated to the herd level to calculate prevalence of heifers within herds with IMI status before and within 5 d following calving, and within-herd cumulative incidence of CM within 14 d of calving. Results from samples and measurements taken >5 d postpartum that were for the routine postpartum sampling >14 d for CM cases were discarded for analysis. Breed of heifer was defined as the predominant parentage breed if greater than 11/16th (Friesian or Jersey), and all other breeds including crossbreds were classified as "other." In calculating proportions, samples that were either contaminated or not collected were not counted in the denominator. Records of gland function and presence or absence of thelitis at 3 mo after the start of the calving program were not analyzed from quarters that did not have a sample collected within 5 d following calving or were recorded with thelitis before or within 5 d of calving. Individual herd test records from heifers <10 d from date of calving or within 14 d of diagnosis of CM were excluded from analysis of ISCC.

Statistical Analysis

Descriptive analysis was carried out for measures of IMI and CM at quarter, heifer, and herd levels. Adjustment of *P*-values for multiple comparisons (when used) was by Holm correction. Prevalence of quarter IMI by day relative to calving was plotted using a loess-smoothed curve. Because heifers within individual herds were sampled precalving at one visit only, but they calved over a subsequent 2- to 14-wk period, an approximate description of prevalence of IMI in terms of days relative to calving could be made. Days from precalving and postcalving sampling to day of individ-

Table 1. Names and definitions of quarter-level results from microbiological testing of milk samples

Summary measure	Definition
Major ¹ pathogen positive	One or more major pathogen species isolated at 1 sampling
Minor ² pathogen positive	One or more minor pathogen species isolated at 1 sampling
Pathogen negative	No major or minor pathogen species isolated at 1 sampling
Null	No sample collected or sample contaminated at 1 sampling
IMI same	One or more same bacteria isolated both pre- and postcalving
New IMI	A bacteria isolated postcalving was not isolated precalving

¹*Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

²CNS, *Corynebacterium* spp., undifferentiated gram-negative rods, undifferentiated gram-positive rods, and yeasts.

ual calving were grouped into quintiles for χ^2 test for trend in proportions (prevalence) of IMI relative to day of calving. The crude daily hazard (risk) of diagnosis of first case of CM within 14 d following calving was plotted separately for heifers and all other parity groups combined, and differences between survival curves for groups tested by log-rank test.

Data from this study were of a hierarchical nature (quarters nested within heifers, in turn nested within herds), and observations at the lower 2 levels of measurement could not be considered independent of others within the same level. Analysis of such data with methods accounting for correlation between outcomes was necessary to avoid overly optimistic interpretations of probability values for association and biased point estimates (Dohoo et al., 2003). Hence, for dichotomous outcomes (reduced quarter size or function, thelitis, ISCC >200,000 cells/mL at herd tests, and risk of premature removal from the herd), linear mixed logistic regression models were fitted with multivariate normal random effects and random intercepts by penalized quasi-likelihood run within R (R Development Core Team, 2005). Continuous outcome measures (milk yield and milk solids production at the first herd test and average of 3 to 4 herd tests in lactation) were modeled using REML methods in the same software. For outcome measures recorded at the quarter level (e.g., quarter function or thelitis), 3-level models were used with both heifer and herd as random effects, and for those models with outcomes recorded at the heifer level (e.g., milk production, ISCC, and risk of removal), 2-level models were used with herd as a random effect. The choice of correlation structures for errors was made using biological reasoning and likelihood ratio test for improvement in model fit. Thus, autoregressive type 1 structure was used for repeated measures of continuous outcomes (e.g., milk production measures and log ISCC) and compound symmetry used for binary outcomes (e.g., quarters within heifers) or continuous measures made only once per heifer (milk production measures at first production recording in lactation).

Three-level generalized linear mixed regression models may be represented as

$$(g)\mathbf{Y}_{ijk} = \beta_0 + \beta_1 \mathbf{X}_1 + \beta_2 \mathbf{X}_2 \\ + \dots \beta_n \mathbf{X}_n + \mu_{\text{heifer}(j)} + \mu_{\text{herd}(k)} + \varepsilon_{ijk}$$

where (g) refers to the link function (logit for logistic models), $\mathbf{Y}_{ijk}$ is the outcome variable, β s are the model coefficients, $\mathbf{X}$ s are the variables included in the models (prior bacteriological status and days from calving to sampling when postcalving IMI status used as a covariate), j refers to the heifer, k refers to the herd, and i to the i th quarter in the j th heifer in the k th herd, and the random effects are independent and normally distributed: $\mu_{\text{heifer}(j)} \sim N(0, \sigma^2_{\text{heifer}})$, $\mu_{\text{herd}(k)} \sim N(0, \sigma^2_{\text{herd}})$, $\varepsilon_{ijk} \sim N(0, \sigma^2)$.

Heifer-level responses were modeled at 2 levels where the residual error was heifer and herd was a random effect. For production outcome models, predictor variables included were postcalving IMI and CM status (1/0), breed, days from calving to test date, and EBV as fixed effects. Individual SCC were natural log-transformed for analysis, and then back transformed for display of results. The variable “days from calving to test” was fit as single continuous covariate for models of measures of milk yield and milk solids production, because a polynomial term did not improve model fit. Nevertheless, a quadratic term was used for modeling average-lactation ISCC. Plots of residuals from all final models were examined to detect unusual patterns, but none were detected.

The intraclass correlation coefficient (ICC) estimates the resemblance among observations within a class (cluster) and may vary between 0 (independent) and 1 (totally correlated). It provides an indication of the infectiousness of an organism or similarity in susceptibility to disease because of common factors within the cluster and is required for estimating sample size in cluster surveys. Estimates of the ICC for a particular outcome at different levels of aggregation reflect the

contribution of those levels to the overall variance, with the expectation that interventions targeted at a particular level with the highest ICC might have the greatest impact on the outcome (Dohoo et al., 2001). For binary data, a maximum likelihood method for point estimate and delta method for confidence intervals were calculated for each level of clustering using the method reported by Zou and Donner (2004).

Initially, unconditional associations between dichotomous outcomes and explanatory variables were examined using crude hazard ratios and incidence risk ratios for number of events, all evaluating categorical risk factors. Univariate ordinary logistic regression was used for continuous risk factors. Variables with associations significant at probability values ≤ 0.20 were considered for inclusion in multivariable models. Wald tests were used to assess the significance of adding fixed effect terms in a forward stepwise way, and those with Wald test P -values ≤ 0.05 and variables known or suspected a priori as confounders (e.g., breed, DIM at milk test, and genetic breeding worth) were included in the final model. Other variables were considered for evidence of confounding and included in a final model if they caused $>10\%$ change in a regression coefficient due to their inclusion in the model, but there were none. All first-order interaction terms were tested in a forward stepwise manner and considered for the final model if significant ($P \leq 0.05$), but none were found.

Because most outcomes were not rare, the odds ratio as measure of association could not be interpreted as multiplicative measures of risk and was not considered appropriate. Instead, incidence risk ratios (IRR) were used because they accurately describe the multiplicative risk of an outcome occurring for a given level of exposure compared with a reference exposure level. Incidence risk ratios and their confidence intervals were not obtainable directly from standard logistic regression models using the canonical logistic link, but were instead estimated from mixed logistic regression models using the log link (McNutt et al., 2003). Predicted population average estimates of continuous production outcomes were estimated from final models, using the most frequent categorical variable (Friesian breed) and the mean values of the other covariates as predictors.

Data were recorded in an Access (Microsoft Corp., Redmond, WA) database. Statistical significance for tests was declared at $P \leq 0.05$, and confidence intervals reported for a 95% of range of values.

RESULTS

Heifers lost to follow up after enrollment included 11 heifers that did not calve (9 were not pregnant and 2 were culled for other reasons); a further 31 heifers

(including 1 that died of acute mastitis) that were not submitted by farmers for sampling within 5 d following calving; and 1 heifer that was destroyed due to persistent obstetric paralysis before 14 d postcalving. Thus, 666 heifers were sampled within 5 d following calving, and 696 heifers were considered at risk for CM within 14 d of their calving date.

Quarter-Level Microbiological Results

Precalving quarter samples were taken 41 ± 16.4 d before individual calving date. The prevalence of infected glands precalving was 18.5% (Table 2), with CNS (13.5%) and *Strep. uberis* (2.8%) being the most prevalent isolates. Other bacterial isolates were infrequent and grouped together as "other." Quarter IMI prevalence precalving was significantly ($P < 0.01$) higher for minor compared with major pathogens. Dual infections in quarters precalving were uncommon; $<2\%$ of quarters had 2 different isolates identified in the same quarter. Forty of 47 dual infections were in combination with *Strep. uberis*, with a small number each of other possible combinations.

Postcalving samples were taken 2.0 ± 1.2 d after individual calving date. The quarter-level prevalence of IMI increased from 18.5% precalving to 21.5% postcalving (Table 2). For quarters with paired samples pre- and postcalving, absolute prevalence increased by 2.9%. This change was due to a significant increase in major pathogen prevalence (particularly *Strep. uberis*) from 3.9 to 11.6% and a 4.7% decline in minor pathogen prevalence (mainly CNS). The postcalving prevalence of major pathogens was significantly higher ($P = 0.05$) than that of minor pathogens. Other pathogens were infrequently isolated, and dual isolates were recovered from only 2% of quarters involving principally minor pathogens isolated in conjunction with *Strep. uberis* or *Strep. dysgalactiae*.

Minor pathogen prevalence was consistently higher than major pathogen prevalence precalving ($P < 0.05$ for all periods) and increased approaching calving ($P < 0.01$; Figure 1). Major pathogen prevalence remained low and did not differ significantly over the precalving sampling period ($P = 0.38$). Prevalence of minor pathogen IMI did not change for the specific interval between -27 and -9 d precalving and the day of calving (difference = 1.9%), but major pathogen prevalence increased [difference = 21.4%, confidence interval (CI) for difference: 16.6 to 26.5%] for the same period. Following calving, both major and minor pathogen prevalence declined rapidly after day of calving to $<7\%$ by d 5 following calving ($P < 0.01$ for both pathogen categories), and there was no difference in prevalence between pathogen categories within each period ($P > 0.20$).

Table 2. Count (%) of results of bacteriological sampling from heifer quarters and clinical mastitis (CM) quarters during prepartum and postpartum periods (days relative to calving) in 708 pasture-grazed dairy heifers

Item	Prevalence of IMI								Cumulative incidence of CM			
	d -127 to -9				d 0 to 5				d 0 to 14			
	Isolate 1		Isolate 2		Isolate 1		Isolate 2		Isolate 1		Isolate 2	
Quarters (n)	2,832				2,664				195			
CNS	381	(13.5)	45	(1.6)	258	(9.7)	53	(1.9)	15	(7.7)	6	(3.1)
Contaminated	75	(2.6)			12	(0.5)			—	—		
<i>Corynebacteria</i> spp.	7	(0.2)	1	(0.0)	2	(0.0)						
<i>Escherichia coli</i>	7	(0.2)	1	(0.0)	14	(0.5)	3	(0.1)	7	(3.6)	2	(1.0)
<i>Staphylococcus aureus</i>	12	(0.4)			16	(0.6)			5	(2.6)		
<i>Streptococcus dysgalactiae</i>	2	(0.1)			10	(0.4)			6	(3.1)		
<i>Streptococcus agalactiae</i>	1	(0.0)			—	—			—	—		
<i>Streptococcus uberis</i>	78	(2.8)			267	(10.0)			125	(64.4)		
Other ¹	14	(0.5)			2	(0.1)			0	0		
No growth	2,208	(78.0)			2,075	(77.9)			36	(18.6)		
No sample	47	(1.7)			8	(0.3)			1	(0.5)		
Major pathogen	107	(3.9)*, ^a			307	(11.6)*, ^a			143	(73.3)		
Minor pathogen	395	(14.6)*, ^b			262	(9.9)*, ^b			15	(7.7)		
All pathogens	502	(18.5)*			569	(21.5)*			158	(81.0)		

^{a,b}Prevalence within columns differ ($P \leq 0.05$).

¹*Enterococcus* spp. (n = 1), gram-negative rods (n = 5), gram-positive rods (n = 2), *Klebsiella* (n = 1), *Pasteurella* spp. (n = 4), and *Proteus* spp. (n = 1)

*Prevalence within rows differ ($P \leq 0.05$).

Clinical mastitis was diagnosed in 195 quarters from 2,784 quarters at risk within 14 d following calving (cumulative incidence = 0.07; CI = 0.06 to 0.08). *Streptococcus uberis* was the most commonly isolated bacteria

from quarters with CM (64.4% of CM cases including those with no growth results), and in decreasing percentages CNS (7.7%), and *E. coli*, *Strep. dysgalactiae*, and *Staph. aureus* (3 to 4% each). No bacterial isolates

Figure 1. Smoothed prevalence of quarter IMI by day relative to calving for major (—■—) and minor (—▲—) pathogens (A) precalving and (B) postcalving, in 708 pasture-grazed dairy heifers.

Table 3. Count (%) of results of paired bacteriological samples from quarters pre- and postcalving in 708 pasture-grazed dairy heifers

Pecalving result	Observations precalving (n)	Postcalving (0 to 5 d) result			
		Same isolate or result		New isolate	
CNS	357	117	(33)*	63	(18)
<i>Corynebacterium</i> spp.	7	0	(0)	3	(43)
<i>Escherichia coli</i>	6	0	(0)	1	(17)
No growth	2,058	1,755	(85)	303	(15)
<i>Staphylococcus aureus</i>	10	5	(50)	1	(10)
<i>Streptococcus dysgalactiae</i>	2	2	(100)	0	(0)
<i>Streptococcus agalactiae</i>	1	0	(0)	0	(0)
<i>Streptococcus uberis</i>	75	44	(59)*	3	(4)
Other ¹	15	1	(67)	3	(20)
Total	2,530	1,924	(76)	377	(15)

¹*Enterococcus* spp. (n = 1), gram-negative rods (n = 5), gram-positive rods (n = 2), *Klebsiella* spp. (n = 1), *Pasteurella* spp. (n = 4), and *Proteus* spp. (n = 1).

*Proportions within column differ ($P \leq 0.05$).

were recovered from 18% of samples submitted for culture from cows diagnosed with CM, and no samples were defined as contaminated. Dual isolates from the same quarter with CM were diagnosed in only 8 (4%) cases; in these, CNS or *E. coli* were isolated in conjunction with *Strep. uberis*. Of the 569 quarters with any pathogen sampled within 5 d following calving, 96 were diagnosed with CM in the same quarter at the same time (16.9%; CI = 14.0 to 20.2%).

Paired bacteriological results from the same quarters were compared over time after data was coded according to the definitions in Table 1. Overall, 1,755 of 2,530 quarters (69.9%) had samples pre- and postcalving that were both negative (Table 3). Of all quarters with pathogens isolated precalving, 35.8% had the same bacteria isolated postcalving; conversely, then, 64.2% of isolates self-eliminated. Yet, significantly more major pathogens (mainly *Strep. uberis*) than minor pathogens (mainly CNS) persisted over this period. Almost all (46 of 48 or 96%) quarters diagnosed with CM between 6 to 14 d following calving had the same isolate diagnosed at a prior sampling within 5 d of calving. Four hundred seventy-three quarters had IMI postcalving that were subclinical at the time of sampling, and of these 48 (10.1%) were subsequently diagnosed as CM within 14 d following calving.

Prevalence of IMI by quarter position did not differ significantly precalving for either minor or major pathogens (Table 4). Postcalving, there was a higher prevalence of major ($P < 0.01$), but not minor pathogens, in rear vs. front quarters, and CM was more frequently ($P < 0.01$) diagnosed in rear compared with front quarters.

Heifer-Level Results

Pecalving, 268 of 708 (38%) of heifers had one or more quarters infected with bacteria, with 182 (26%)

and 86 (12%) of heifers diagnosed with minor and major pathogen IMI, respectively. Significantly ($P < 0.01$) more heifers had an IMI postcalving (323 of 666, prevalence = 49%; difference = 11%). Minor pathogen prevalence decreased ($P < 0.01$) 11% to 103 of 666 heifers (16%), and there was a concurrent increase ($P < 0.01$) in prevalence of heifers with a major pathogen (n = 220, prevalence = 33%, postcalving, difference = 20.9%). Precalving, bacteria were isolated from 1, 2, 3, and 4 glands in 126 (18%), 75 (11%), 42 (6%), and 25 (4%) heifers, respectively. Postcalving, IMI were isolated from 1, 2, 3, and 4 glands respectively in 170 (25.6%), 80 (12.0%), 53 (8.0%), and 20 (3.0%) of heifers, respectively.

Clinical mastitis was diagnosed in 163 of 696 heifers (cumulative incidence = 0.234) within 14 d following calving. One hundred forty (20%), 17 (3%), 3 (<1%), and 3 (<1%) heifers had 1, 2, 3, or 4 quarter CM cases, respectively, diagnosed concurrently. The daily hazard or risk of diagnosis of CM in early lactation (Figure 2) was higher ($P < 0.01$) in heifers than in greater parity cows, and from inspection of the confidence intervals for the curves (not shown), this period of greater risk occurred from d 1 to 7 of lactation, particularly on the day following calving when the risk in heifers was approximately 3 times that of older cows. A log-rank test confirmed that survival curves to first case of CM were significantly different between groups ($P < 0.01$). One hundred heifers had both samples for CM and routine postcalving sampling taken at the same visit. Of the 83 heifers with 1 quarter diagnosed with CM, 41 (50%) had 1 or more additional quarters with any pathogen IMI, and of the 15 heifers diagnosed with CM in 2 quarters, 5 (38%) had 1 or more additional quarters with IMI.

Herd-Level Results

Wide variation existed between herds for all the outcome measures. Prevalence of precalving IMI of heifers

Table 4. Count (%) of IMI and clinical mastitis by quarter position pre- and postcalving in 708 pasture-grazed dairy heifers

Sampling period relative to calving	Bacteriological result	Left fore		Left rear		Right fore		Right rear		Total
d -127 to -9	Minor pathogen IMI	90	(13)	101	(15)	96	(14)	108	(16)	395
	Major pathogen IMI	16	(2)	30	(4)	26	(4)	35	(5)	107
	Total quarters sampled	679		681		665		685		2,710
d 0 to 5	Minor pathogen IMI	67	(10)	67	(10)	68	(10)	60	(9)	262
	Major pathogen IMI	40	(6) ^a	107	(16) ^b	38	(6) ^a	122	(19) ^b	307
	Total quarters sampled	660		661		662		661		2,644
d 0 to 14	Clinical mastitis cases	31	(4) ^a	78	(11) ^b	22	(3) ^a	64	(9) ^b	195
	Total quarters at risk	697		697		697		697		2,788

^{a,b}Proportions within rows with different superscripts differ ($P \leq 0.05$).

within herd was $37 \pm 12\%$, that for major pathogens was $12 \pm 7\%$, and that for minor pathogens was $25 \pm 10\%$. Postcalving, the within-herd prevalence of heifers with an IMI was $47 \pm 16\%$, that for major pathogens was $33 \pm 14\%$, and that for minor pathogens was $14 \pm 9\%$. The within-herd cumulative incidence of CM within 14 d following calving was $25 \pm 12\%$.

Clustering of IMI and CM at Heifer and Herd Levels

The estimated ICC for heifer level outcomes were, in each case, greater than for those at the herd level (Table 5). The heifer-level ICC estimates were <0.20 and significantly different from 0, except for precalving infec-

tion with minor pathogens (ICC = 0.26), but significant herd-level clustering existed for major pathogen IMI postcalving and CM.

Effects on Productivity

Associations between postcalving IMI and CM and diagnosis of quarters with reduced function or thelitis at 3 mo following the end of the seasonal calving period are shown in Table 6. Ten quarters were completely nonfunctional, and these were included with reduced function quarters for analysis because of their low prevalence. The risk of reduced function was 13% higher ($P < 0.01$) in quarters with, than without, major pathogens isolated postcalving, and 17% higher ($P < 0.01$) in quarters diagnosed with, than without, CM within 14 d following calving (IRR of 9.4 and 9.1, respectively). The risk of thelitis was significantly higher ($P < 0.01$) in quarters with a major pathogen isolated postcalving (incidence risk ratio = 2.3), but was similar in quarters with CM compared with normal quarters. The overall incidence of thelitis was low ($<2\%$ of teats).

Milk yield at first production recording and average daily milk yield over the entire lactation were 0.6 and 0.7 kg higher, respectively ($P = 0.04$ and $P < 0.01$, respectively) in heifers with IMI due to minor pathogens postcalving compared with heifers with no pathogens isolated, after adjustment for effects of breed, day of lactation at test date, and genetic breeding worth (Table 7). Other measures of milk yield and milk solids production were not significantly associated with postcalving bacteriological or CM status. Isolation of major pathogens or any pathogens postcalving in one or more quarters, or CM within 14 d of calving were significantly associated with geometric mean first herd-test SCC, equivalent to increases of 22×10^3 ($P < 0.01$), 17×10^3 ($P < 0.01$), and 12×10^3 cells/mL ($P = 0.02$), respectively. Isolation of a minor pathogen alone postcalving from one or more

Figure 2. Instantaneous hazard (daily risk) of clinical mastitis in pasture-grazed primiparous heifers (—■—) or multiparous cows (—▲—) relative to individual calving date.

Table 5. Intraclass correlation coefficients (ICC) estimated for IMI and clinical mastitis outcomes in 708 pasture-grazed dairy heifers

Sampling period relative to calving	Bacteriological result	Level of clustering	ICC	SE	P-value
d -127 to -9	Minor pathogen IMI	Heifer	0.26	0.01	<0.01
		Herd	0.01	0.03	0.16
	Major pathogen IMI	Heifer	0.15	0.01	<0.01
		Herd	0	0.08	0.48
d 0 to 5	Minor pathogen IMI	Heifer	0.13	0.01	<0.01
		Herd	0.02	0.03	0.12
	Major pathogen IMI	Heifer	0.15	0.02	<0.01
		Herd	0.04	0.02	<0.01
d 0 to 14	Clinical mastitis cases	Heifer	0.08	0.02	<0.01
		Herd	0.04	0.02	<0.01

quarters was not significantly associated with ISCC. Culture of major pathogens from one or more quarters postcalving was associated with an increased risk ($P < 0.05$) of heifer ISCC $>200 \times 10^3$ cells/mL throughout lactation (IRR = 1.95, CI = 1.65 to 2.32, incidence risk = 0.152 vs. 0.078) and at test 1 (IRR = 2.40, CI = 1.28 to 4.48), test 2 (IRR = 2.86, CI = 1.63 to 5.03), test 3 (IRR = 2.96, CI = 1.67 to 5.25), and test 4 (IRR = 1.37, CI = 1.01 to 1.87). Diagnosis of CM in one or more quarters within 14 d of calving was associated with an increased risk ($P < 0.01$) of heifer ISCC $>200 \times 10^3$ cells/mL at test 1 only (IRR = 2.36, CI = 1.32 to 4.21). Culture of minor pathogens postcalving from one or more quarters was not associated with risk of heifer ISCC $>200 \times 10^3$ cells/mL at any subsequent herd test.

During the entire lactation, 87 enrolled heifers (12%) were removed from the herds. The most common reason given for removal was failure to conceive following the seasonal breeding program ($n = 65$). Mastitis was given as a reason for removal for only 6 heifers (<1%). But, isolation of major pathogens from one or more quarters of heifers postcalving significantly increased ($P = 0.02$) the overall risk of premature culling from the dairy

herd (crude incidence risk = 0.15 for major pathogen-positive heifers and 0.10 for major pathogen-negative heifers, adjusted IRR = 1.6, CI = 1.1 to 2.3) through an indirect effect on risk of infertility.

DISCUSSION

The pattern of prevalence and bacterial species involved in peripartum IMI in pasture-grazed dairy heifers had some similarities, but important differences from that reported in other countries and under different management systems. In agreement with findings from other studies, the prevalence of CNS at the quarter level was highest precalving (13.5%), and declined as lactation commenced (9.7%). Reported quarter prevalence estimates for CNS were 14 to 6% (Oliver and Sordillo, 1988) and 27 to 12.1% (Aarestrup and Jensen, 1997) for 1 to 2 wk precalving, and 1 to 2 wk postcalving, respectively. However, in this study, IMI before calving due to *Staph. aureus* was infrequent (0.4% of quarters), in contrast to 14.9% by Trinidad et al. (1990), but similar to the 0.5% reported by Aarestrup and Jensen (1997). The precalving quarter-level prevalence of IMI

Table 6. Associations between postcalving IMI type and clinical mastitis (CM) and reduced quarter function and teat thelitis in mid lactation in 708 pasture-grazed dairy heifers

Exposure variable	Exposed ¹	Observations (n)	Reduced function ²			Thelitis ²		
			IR	IRR	IRR CI	IR	IRR	IRR CI
Minor IMI	1	261	0.027	0.9	(0.4, 1.9)	0.015	0.9	(0.3, 2.5)
	0	2,355	0.031			0.017		
Major IMI	1	300	0.147	9.4	(6.2, 14.4)	0.033	2.3	(1.1, 4.6)
	0	2,316	0.016			0.015		
Any IMI	1	561	0.091	6.4	(4.1, 10.1)	0.025	1.7	(0.9, 3.2)
	0	2,055	0.014			0.015		
Clinical mastitis	1	191	0.188	9.1	(6.1, 13.5)	0.031	2.1	(0.9, 4.8)
	0	2,552	0.021			0.015		

¹1 = exposed to risk; 0 = not exposed to risk.

²IR = incidence risk; IRR = incidence risk ratio; IRR CI = incidence risk ratio 95% confidence interval.

Table 7. Predicted population milk volume, milk solids production, and SCC in 708 pasture-grazed dairy heifers of differing postcalving bacteriological and clinical mastitis status

Exposure variable	Exposure level ¹	Milk volume (kg)		Milk solids (kg)		ISCC ² (× 10 ³)	
		Observations (n)	Mean	Observations (n)	Mean	Observations (n)	Mean ³
First production test ⁴							
Minor pathogen	1	94	18.18*	94	1.39	90	53.86
	0	514	17.59	514	1.36	501	55.27
Major pathogen	1	203	17.64	203	1.35	193	70.46**
	0	405	17.68	405	1.37	398	48.89
Any pathogen	1	297	17.80	297	1.36	283	65.23**
	0	311	17.52	311	1.36	308	47.86
Clinical mastitis	1	148	17.80	148	1.38	139	66.05*
	0	481	17.63	481	1.36	473	53.88
Lactation mean							
Minor pathogen	1	378	14.18*	378	1.13	373	50.58
	0	2,013	13.54	2,013	1.09	1,996	49.98
Major pathogen	1	806	13.52	805	1.08	793	61.18**
	0	1,585	13.66	1,585	1.10	1,576	45.04
Any pathogen	1	1,184	13.70	1,183	1.10	1,166	57.95**
	0	1,207	13.52	1,207	1.10	1,203	43.85
Clinical mastitis	1	586	13.64	585	1.10	575	55.60*
	0	1,896	13.62	1,896	1.09	1,885	48.96

¹1 = exposed to risk, 0 = not exposed to risk.²Individual SCC.³Geometric mean.⁴First milk production test in lactation* $P \leq 0.05$; ** $P \leq 0.01$ for differences between exposure levels.

for all pathogens in this study of 18.5% is lower than that of other studies (Trinidad et al., 1990). Another difference in this study compared with others was the low prevalence of coliform and mixed IMI. Less than 0.5% of IMI both pre- and postcalving were due to gram-negative bacteria, compared with 3% by Oliver and Sordillo (1988). Prevalence estimates by Aarestrup and Jensen (1997) for coliform bacteria, however, were less than 1% throughout the peripartum period. Intramammary infection with coliform bacteria is a recognized problem in dairy cattle managed under confinement systems, but coliform bacteria were isolated from less than 5% of CM cases in all age cows under pasture-grazing systems in New Zealand (McDougall, 1998) and from 0.7% of heifers within 5 d of calving (Pankey et al., 1996). An association between low prevalence of coliform CM and pasture grazing was reported in Dutch dairy herds (Olde Riekerink et al., 2007). Reasons for the low prevalence of coliform IMI and the low incidence of coliform CM in pasture-based compared with confinement dairy systems are not defined, but may include the high exposure of pasture-grazed animals to *Strep. uberis* on areas of high stock movement (Lopez-Benavides et al., 2005) and competitive exclusion by this bacteria of coliforms, or differences in pathogen virulence between systems.

An important finding of this study is that *Strep. uberis* was by far the most common major pathogen caus-

ing IMI both pre- and postpartum and CM in dairy heifers in New Zealand. *Streptococcus uberis* was isolated from 72 and 87% of major pre- and postcalving infections, and 65% of CM cases. Precalving *Strep. uberis* prevalence of 2.8% of quarters was slightly lower than the 3.4% reported by Oliver and Sordillo (1988), and similar to the 2% found by Aarestrup and Jensen (1997). Still, postcalving *Strep. uberis* quarter level prevalence increased almost 4-fold to 10%, whereas others have reported relative increases of 2.2-fold (Oliver and Sordillo, 1988) and 1.1-fold (Aarestrup and Jensen, 1997). Reasons for the high relative importance of *Strep. uberis* are not known, but were suggested to be associated with pasture-grazing (Olde Riekerink et al., 2007) and may be related to the high exposure to this bacteria (Lopez-Benavides et al., 2005). The finding of a high postcalving *Strep. uberis* prevalence is consistent with other studies in terms of relative importance, but is much higher than the prevalence reported in a similar population by Pankey et al. (1996). *Streptococcus uberis* is the most common isolate from IMI and CM in all-parity cows early postpartum in New Zealand dairy systems (McDougall, 1998), suggesting a high level of exposure in all parity groups under the pasture grazing systems common in New Zealand.

Comparing quarter infection prevalence over time allowed description of the period of highest infection rate. Approximately 80% of new major IMI occurred in

the last 2 to 3 wk of gestation. Major IMI prevalence did not differ significantly over the precalving study period, was maximal on the day of calving, and then declined rapidly from approximately 20 to <7% within 5 d postcalving. Therefore, the majority of new *Strep. uberis* infections would be occurring in the final 2 wk of gestation in pasture-grazed dairy heifers. This was not likely due to calendar date as the calving dates varied from July to September. Thus, the increase in incidence of new infection appears related to proximity to date of calving, not some climatic effect through this period.

Data from repeated samplings over time and of all quarters added important information on the patterns of IMI and CM over the peripartum period. The relatively high proportion (33% for CNS and 59% for *Strep. uberis*; Table 3) of precalving IMI isolated a second time in early lactation shows the importance of controlling these infections before they cause problems at calving. Over the first 5 d of lactation, only 17% of all IMI showed clinical changes, meaning that for every CM case, about 6 other IMI were undiagnosed. Half of the heifers with CM in 1 quarter had additionally 1 or more other quarters with a subclinical IMI. Failure to diagnose subclinical infections may mean that milk from these quarters will be of lower quality for processing, may increase the risk of violations of SCC standards, and may transmit infection to other quarters and animals in the herd. Only a small proportion (10%) of subclinically infected quarters within 5 d after calving was diagnosed with CM 6 to 14 d postpartum. A proportion of these subclinical IMI may have persisted into lactation, as suggested by the higher risk of ISCC >200,000 following major pathogen IMI, but most IMI did not lead to CM. Almost all the quarters cases of CM 6 to 14 d postcalving had the same isolate 0 to 5 d postcalving, reinforcing the importance of controlling new infections at or immediately before the time of calving to reduce CM and improve milk quality in later lactation.

The cumulative incidence of peripartum CM differed from those described in other countries and management systems. The 23% cumulative incidence of CM in heifers within 14 d following calving observed was higher than the 12% of heifers reported by Barnoun and Chassagne (2001), and the 8.1% of heifers reported by Pankey et al. (1996) within 5 d following calving. The finding that 7.7% of quarters with CM isolated CNS species alone is similar to that reported by Pankey et al. (1996). Although CNS are regarded as minor pathogens (Timms and Schultz, 1987) that rarely cause clinical signs or substantial increases in ISCC, they should not be disregarded as potential causes of CM in peripartum heifers. The finding of a higher hazard of early postpartum CM in heifers compared with cows

supports Barkema et al. (1998). This age-related difference suggests that different risk factors for CM operate for heifers and that mastitis in heifers may have to be addressed with alternative prevention and control efforts to those against mastitis in cows.

There were differences in the distribution of IMI and CM between front and rear quarters and the pattern over time, which may have implications for their etiology. Because minor pathogens (principally CNS) were isolated in equal proportions between front and rear quarters (Table 4), these observations support the hypothesis that these are opportunistic skin pathogens (Harmon and Langlois, 1989) that are present in similar concentrations on all teats and only invade glands with open teat canals. In contrast, major environmental pathogens (principally *Strep. uberis*) preferentially infected rear quarters of heifers postcalving, supporting the findings of Barkema et al. (1997), and those of McDougall (1998) for CM cases in all age groups in early lactation.

Staphylococcus aureus is usually considered a contagious pathogen spread between cows in lactation during the milking process. Nevertheless, this classification does not fit our finding of *Staph. aureus* IMI before the first milking in heifers. Others (Roberson et al., 1994) have drawn attention to the risk of introduction of *Staph. aureus* to the adult milking herd by heifers with infections on multiple body sites (including teat skin and external orifices). Hence, biosecurity measures should be considered before introduction of purchased or self-sourced replacements to pasture-grazed herds in which control programs for *Staph. aureus* are in place.

Data from this study show that 38% of pasture-grazed heifers had one or more quarters with IMI in the precalving sampling period, which is much lower than the 97% found by Trinidad et al. (1990). Although caution must be taken in comparing bacteriological results from different studies due to differing methodologies, the limited available data suggest that the prevalence of IMI precalving in pasture-grazed dairy heifers in New Zealand is relatively low compared with heifers in other production systems. Nevertheless, a prevalence of 38% is high in absolute terms, suggesting significant exposure to pathogens before milking and a need for specific heifer control programs.

Herds varied numerically in their prevalence of IMI and incidence of CM, despite all using similar pasture-grazing management systems. Moreover, because an aim of this study was to describe quarter- and heifer-level, and not herd-level, patterns of IMI and CM, insufficient herds and in some cases heifers within herds were enrolled to make precise estimates of herd level measures. The estimates of ICC between quarters showed low to moderate clustering of IMI and CM mea-

tures (Table 5) at heifer level and small significant clustering at the herd level for major pathogen IMI postcalving and CM. These values were similar to those found by Barkema et al. (1997) for cows in lactation. For each outcome measure, clustering at the heifer level was several-fold greater than at the herd level. This may be interpreted that successful interventions targeting the individual animal are likely more rewarding than those aimed at herd-level management. Yet, with increasing herd sizes in New Zealand, opportunities for individual animal interventions are few, and disease control programs must usually operate at the group level. Hence, new studies are required to understand herd-level risk factors for heifer IMI and CM under pasture-based systems.

Estimation of the impact of IMI and CM on heifer productivity and longevity are important for economic analysis of proposed preventive programs. Many factors must be considered in such analyses, but important components are the effects of disease on production and longevity in the herd. Infection of a quarter with a major pathogen postcalving increased the risk 9-fold of that same quarter having reduced function subsequently (Table 6). This agrees with the finding of Waage et al. (2000), who found 25% of quarters with CM, subsequently had one or more nonfunctional quarters (19% in this study), although a feature of many cases in their study was infection with *Arcanobacter* spp. and *Staph. aureus*. Both of these are known to cause chronic and sometimes severely damaging infections, but were found to be absent or rare, respectively, in this study. Similar to their findings, thelitis occurred with higher prevalence in quarters with a major pathogen isolated postcalving. Although quarters with reduced function, asymmetry, or thelitis did not have direct economic consequences measured, these quarters are unsightly and may increase the difficulty of managing affected heifers.

Estimation of the effects of IMI and CM on milk production is not straightforward. A central problem is the confounding effect of milk production on risk of mastitis (Grohn et al., 2004). Cows with higher milk production potential may be at higher risk of mastitis than lower producing cows; hence, milk production measured in the season of CM may not be measurably different from non-CM herd mates after adjusting for other confounders such as age, breed, and days of lactation. In multiparous cows, the previous season's milk production can be used as a covariate to control for this effect, and the estimations made within-cow and between lactations, or production from earlier in lactation can be used as a covariate. However, this is not possible in heifers in their first lactation and when mastitis occurs very early in lactation before the first herd test.

In this study, both milk yield and milk solids production recorded at the first herd test and averaged over the total lactation were used as outcome measures to estimate effect of IMI or CM on productivity (Table 7). Although the frequency of testing in New Zealand herds is low (up to 4 tests per lactation), meaning that estimates of production were imprecise, statistically significant associations may not have been detected. Data from this study found a small but significant positive association between IMI due to a minor pathogen postcalving and milk yield at the first herd test and averaged over the lactation, after adjustment for known confounders. This finding should not be interpreted as meaning that mastitis increased milk production, but more likely supports the belief that heifers with minor pathogen IMI precalving tend to have higher milk production potential. Grohn et al. (2004) found that *Strep. uberis* CM cases in heifers in the first week of lactation caused relatively small and short-term losses in milk production. This supports the finding of this study of no significant decrease in production in heifers with CM postcalving, when *Strep. uberis* was the predominant major pathogen.

Although heifers that had CM or IMI, or both, with a major pathogen or any pathogen early postpartum had significantly increased mean ISCC at first herd test or averaged over the lactation, the increase over the entire season was small. Nonetheless, significant associations existed between heifers with postcalving IMI due to major pathogens or heifers with CM, and subsequent high ISCC ($>200 \times 10^3$ cells/mL). There was significant risk of high ISCC at only the first test following CM cases, whereas ISCC remained high at all tests following IMI with major pathogens postcalving. Myllys and Rautala (1995) reported higher ISCC only at the first herd test following CM cases, and not at subsequent tests in the lactation. The findings suggests that CM may have short-term effects on ISCC in heifers treated with systemic antibiotics, but that untreated subclinical IMI with major pathogens may lead to IMI that persist through the lactation and cause reduced milk quality for that period.

Replacement of cows prematurely removed from the milking herd is costly to the producer, hence, estimating the risk of removal associated with mastitis is important. The finding that heifers with major pathogens isolated 0 to 5 d after calving had a 60% increased risk of removal from the herd during lactation is important. In this study, IMI with major pathogens was diagnosed in one-third of heifers; thus, the population impact of this infection on culling is high. A possible biological reason for this finding is that CM in the postpartum period was associated with inferior subsequent reproductive performance (Chebel et al., 2004).

This study is the first to report on the epidemiology of environmental mastitis, particularly that caused by *Strep. uberis*, in pasture-grazed dairy heifers before and immediately following parturition. It provides information on patterns of IMI in heifers grazed under these management systems and a basis for formulation of preventive programs.

CONCLUSIONS

Results show that patterns of bacterial species involved and prevalence of IMI in pasture-grazed peripartum dairy heifers differ from those in other production systems, and that the cumulative incidence of CM is high in itself and when compared with older cows. Pre-calving IMI was mainly caused by the skin-opportunistic bacteria, CNS, and the quarter prevalence was relatively low. The incidence of new IMI immediately pre-calving by the environmental bacteria *Strep. uberis* was high and it was the predominant pathogen among all IMI and CM cases. Intramammary infection was not associated with a decrease in milk yield or milk solids production, but did increase the risk of premature removal from the herd.

Current mastitis control programs targeting infectious pathogens are not specifically designed for heifer peripartum mastitis. They are unlikely to be successful because the environment, and not other cows, is the reservoir of the major pathogens involved, and new infections are likely occurring before the first milking when existing detection and control measures can be implemented. Novel control programs that reduce new infections due to *Strep. uberis* immediately before calving are required to reduce the incidence of CM in pasture-grazed dairy heifers. Effective control of environmental mastitis apart from the use of intramammary antibiotics and internal teat sealants has not been consistently reported, but because of the preponderance of one bacterial species (*Strep. uberis*), effective preventive measures against this organism (e.g., by vaccination) are likely to have a large population impact on CM in dairy heifers grazed on pasture.

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REFERENCES

- Aarestrup, F. M., and N. E. Jensen. 1997. Prevalence and duration of intramammary infection in Danish heifers during the peripartum period. *J. Dairy Sci.* 80:307–312.
- Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, D. T. Galligan, M. L. Beiboer, and A. Brand. 1997. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. *J. Dairy Sci.* 80:1592–1599.
- Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J. Dairy Sci.* 81:411–419.
- Barnouin, J., and M. Chassagne. 2001. Predictive variables for the occurrence of early clinical mastitis in primiparous Holstein cows under field conditions in France. *Can. Vet. J.* 42:47–53.
- Chebel, R. C., J. E. P. Santos, J. P. Reynolds, R. L. A. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. *Anim. Reprod. Sci.* 84:239–255.
- Dohoo, I., W. Martin, and H. Stryhn. 2003. *Veterinary Epidemiologic Research*. Atlantic Veterinary College Inc., Charlottetown, Canada.
- Dohoo, I. R., E. Tillard, H. Stryhn, and B. Faye. 2001. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Prev. Vet. Med.* 50:127–144.
- Grohn, Y. T., D. J. Wilson, R. N. Gonzalez, J. A. Hertl, H. Schulte, G. Bennett, and Y. H. Schukken. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 87:3358–3374.
- Harmon, R. J., and B. E. Langlois. 1989. Mastitis due to coagulase-negative *Staphylococcus* species. *Agri-Practice* 10:29–34.
- Hogan, J. S., R. N. Gonzalez, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, and K. L. Smith. 1999. *Laboratory Handbook on Bovine Mastitis*. National Mastitis Council Inc., Madison, WI.
- Lopez-Benavides, M. G., J. H. Williamson, R. T. Cursons, S. J. Lacy-Hulbert, and M. W. Woolford. 2005. *Streptococcus uberis* population dynamics in the New Zealand pastoral dairy farm. Pages 649–655 in 4th IDF International Mastitis Conference. H. Hogeveen, ed. Wageningen Academic Publishers, Maastricht, the Netherlands.
- McDougall, S. 1998. Efficacy of two antibiotic treatments in curing clinical and subclinical mastitis in lactating dairy cows. *N.Z. Vet. J.* 46:226–232.
- McNutt, L. A., C. Wu, X. Xue, and J. P. Hafner. 2003. Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am. J. Epidemiol.* 157:940–943.
- Myllys, V., and H. Rautala. 1995. Characterization of clinical mastitis in primiparous heifers. *J. Dairy Sci.* 78:538–545.
- Olde Riekerink, R. G., H. W. Barkema, and H. Stryhn. 2007. The effect of season on somatic cell count and the incidence of clinical mastitis. *J. Dairy Sci.* 90:1704–1715.
- Oliver, S. P., and L. M. Sordillo. 1988. Udder health in the periparturient period. *J. Dairy Sci.* 71:2584–2606.
- Oltenu, P. A., and I. Ekesbo. 1994. Epidemiological study of clinical mastitis in dairy cattle. *Vet. Res.* 25:208–212.
- Pankey, J. W., P. A. Drechsler, and E. E. Wildman. 1991. Mastitis prevalence in primigravid heifers at parturition. *J. Dairy Sci.* 74:1550–1552.
- Pankey, J. W., P. B. Pankey, R. M. Barker, J. H. Williamson, and M. W. Woolford. 1996. The prevalence of mastitis in primiparous heifers in eleven Waikato dairy herds. *N.Z. Vet. J.* 44:41–44.
- R Development Core Team. 2005. *R: A language and environment for statistical computing*. 2.2.0 ed. R Foundation for Statistical Computing, Vienna, Austria.
- Roberson, J. R., L. K. Fox, D. D. Hancock, J. M. Gay, and T. E. Besser. 1994. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. *J. Dairy Sci.* 77:3354–3364.

- Timms, L. L., and L. H. Schultz. 1987. Dynamics and significance of coagulase-negative staphylococcal intramammary infections. *J. Dairy Sci.* 70:2648–2657.
- Trinidad, P., S. C. Nickerson, and T. K. Alley. 1990. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. *J. Dairy Sci.* 73:107–114.
- Waage, S., H. R. Skei, J. Rise, T. Rogdo, S. Sviland, and S. A. Odegaard. 2000. Outcome of clinical mastitis in dairy heifers assessed by reexamination of cases one month after treatment. *J. Dairy Sci.* 83:70–76.
- Waage, S., S. Sviland, and S. A. Odegaard. 1998. Identification of risk factors for clinical mastitis in dairy heifers. *J. Dairy Sci.* 81:1275–1284.
- Zou, G., and A. Donner. 2004. Confidence interval estimation of the intraclass correlation coefficient for binary outcome data. *Biometrics* 60:807–811.