A multi-level cohort study of the prevalence and incidence of mastitis in pasture-grazed dairy heifers

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Abstract

A longitudinal observational study was undertaken on 708 heifers from 30 pasture-grazed, seasonal calving herds in the Waikato region of New Zealand with the aim of describing their patterns of intramammary infection (IMI) and clinical mastitis (CM) in the peripartum period. Mammary secretion samples were taken from all quarters approximately 3 weeks prior to the planned start of the calving period for heifers in each herd, and within 5 days following calving for bacteriological testing, as were milk samples from all quarters diagnosed with CM within 14 days of calving. Pre-calving IMI was diagnosed in 18.5% of quarters, and of these coagulase negative staphylococci (CNS) were the predominant isolate (13.5% of quarters). Post-calving, CNS predominated (10.6% of quarters), but *Streptococcus uberis* (*S. uberis*) prevalence increased four-fold to 10% of quarters. Prevalence of all pathogens decreased rapidly following calving. Clinical mastitis cases were predominantly associated with *S. uberis*, and the hazard of diagnosis was higher in heifers than other parity groups combined and highest in the first 5 days of lactation. For primiparous cows, SCM was associated with an increased risk of removal from the herd and with high somatic cell count (>200 000 cells/ml), but neither CM nor IMI were associated with reduced milk yield or milk solids production.

Introduction

Heifers (two-year-old primiparous cattle) have a high prevalence of clinical mastitis (CM) and subclinical mastitis (SCM) relative to older animals in herds. Studies in several countries have shown high incidence of CM and intramammary infection (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 has also been reported in heifers before calving (White et al., 1989; Trinidad et al., 1990), and a positive association has been found between pre- and postpartum infection (Oliver and Sordillo, 1988; Matthews et al., 1992; Aarestrup and Jensen, 1997). However, the incidence and prevalence of IMI in peripartum heifers varies between locations and management systems (Fox et al., 1995; Myllys and Rautala, 1995; Waage et al., 1998).

Little data has been published on the epidemiology of peripartum mastitis in pasture-grazed dairy heifers. Pankey et al. (1996) reported 35% of heifers in pasture-grazed herds in New Zealand had one or more quarters diagnosed with IMI within 5 days following calving, and that 8% of heifers had clinical mastitis in the same time period. However, there is no data on the prevalence of IMI before calving in heifers in commercial pasture-grazing farming systems, or on any productivity effects following IMI pre- or post-calving, or following clinical mastitis.

Objective

The main aims of this study were firstly to describe at quarter, heifer and herd level the prevalence of IMI 2-18 weeks prior to, and within 5 days following calving, and the incidence of CM in the first 14 days of lactation in first-calving heifers in commercial pasture-grazed dairy herds. The study also aimed to describe the bacteria involved in heifer peripartum IMI and CM and the repeatability of bacterial isolation across time.

Methods

Between 6 and 27 heifers were randomly selected from a convenience sample of 30 spring-calving dairy herds. Heifers (n=708) were enrolled on one calendar date per herd approximately 4 weeks

before the planned starting date of the seasonal calving period. Heifer breeds were Friesian (n = 291), Jersey (n = 214), and other (predominantly Friesian-Jersey crossbreed) (n = 203). 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 rye grass (*Lolium perenne*) and white clover (*Trifolium repens*) fed *in-situ* with a new area of pasture being offered daily and with small amounts of hay and pasture silage fed also on the grazing area.

At the time of enrolment, each heifer had a single mammary secretion sample taken 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). Duplicate milk samples were collected from all glands within 5 days of calving during routine twice weekly visits to the herds by trained technicians or at the time of diagnosis of CM if this occurred before this planned visit. Duplicate milk samples were also collected from all first cases of CM within 14 days of calving.

Microbiological procedures, diagnosis of IMI, and categorization of results were undertaken using standard methodology (Hogan et al., 1999). Samples from which more than 2 colony types were found 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 *Staphylococcus aureus* (*S. aureus*) where >= 1 colony was recorded as an isolate. Where 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 one of the duplicate samples was contaminated, the results from the uncontaminated duplicate alone were used to diagnose infection.

Bacteriological results were categorized as either major or minor pathogens. Bacterial species classified as major pathogens were *Enterococcus spp.*, *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *S. aureus, Streptococcus agalactiae, Streptococcus dysgalactiae* (*S. dysgalactiae*) and *Streptococcus uberis* (*S. uberis*). Minor pathogens were coagulase negative staphylococci (CNS), *Corynebacterium* spp., undifferentiated gram negative rods, undifferentiated gram positive rods and yeast. Bacteriological results from cases of CM that occurred within 0-5 days following calving were included with the IMI results. CM quarters were also reported separately. 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 the 'major pathogen' status for that sampling occasion. When describing bacteriological results from samples taken at different occasions, the individual quarter bacterial isolates were compared. More than one bacterial isolate was identified from some quarters, and therefore the categories for bacteriological status between samplings for a quarter were not mutually exclusive. Results of bacteriological testing of milk samples were aggregated from quarter to heifer-level and heifer to herd-level using the same major or minor pathogen quarter categorisation definitions.

Descriptive analysis was carried out for measures of IMI and CM at quarter, heifer and herd level. Confidence intervals for proportions and differences of paired proportions were calculated using Wilson's method and method 10 of Newcombe (1998), respectively. Adjustment of P-values for multiple comparisons when used was by Bonferroni correction. Probability of IMI by day relative to calving was explored using non-parametric smoothed logistic regression techniques implemented by Bowman and Azzalini (1997) in their 'sm' software package (Bowman et al., 2005) which is an add-in for "R: A language and environment for statistical computing" (R Development Core Team, 2005) version 2.2.0. This technique calculates probability of a dichotomous outcome (IMI, 0,1) modelling a continuous covariate (days from sampling to calving), and provides graphical output for visual inspection. Because heifers within herds were sampled pre-calving once at one visit, but

they calved over a subsequent 2-14 week period, an approximate description of prevalence of IMI in terms of days relative to calving could be made. The daily hazard (risk) of diagnosis of CM within 14 days following calving in heifers and all other parity cows was described on the basis of a lowess-smoothed Kaplan-Meier survival function (Venables and Ripley, 2002, pg. 353-385).

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

Summary measure	Definition
Major pathogen positive	One or more major pathogen species isolated
Minor pathogen positive	One or more minor pathogen species isolated
Pathogen negative	No major or minor pathogen species isolated
Null	No sample collected or sample contaminated
IMI same	One or more same bacteria isolated both pre- and post-calving
No growth	No major or minor pathogens isolated both pre- and post-calving
New IMI	A bacteria isolated post-calving was not isolated pre-calving
Self-eliminated IMI	A bacteria isolated pre-calving was not isolated post-calving

Data from this study were of a hierarchical nature (quarters nested within heifers, in turn nested within herds), and observations at the lower two levels of measurement could not be considered independent of others within the same level. Mixed regression models were used to account for clustering of data in this study. Generalized linear mixed models were fitted with multivariate normal random effects and random intercepts by penalized quasi-likelihood using the "VR" add-in package for "R: A language and environment for statistical computing" (R Development Core Team, 2005) version 2.2.0, (Venables and Ripley, 2002) for dichotomous outcomes (risk of post-calving IMI and CM), using logistic models. For outcome measures recorded at the quarter-level e.g. quarter IMI and CM status, 3-level models were used with both heifer and herd as random effects.

The ratio of daily hazard of diagnosis of CM of heifers compared to older parity cows was tested using Cox proportional hazards regression with herd as a shared frailty term, represented mathematically as:

$h_i(t|\bullet_j) = \bullet_j h_j(t)\, e^{\bullet X}$

where $\bullet_j$ = frailty for j^{th} herd, $\bullet X = \bullet_1$ age category, and $h_i(t|\bullet_j)$ is the hazard for the i^{th} individual in the j^{th} herd at time t.

Data was recorded in a Microsoft Access database. Statistical significance for tests was taken as P • 0.05, and confidence intervals reported are for a 95% of range of values.

Results

Quarter-level microbiological results

Pre-calving quarter samples were taken on average 41 days (S.D=16.4 days) before calving. The prevalence of infected glands was 18.5 % (CI= 17.1% to 20.0%) pre calving (Table 2). Where a second pathogen was isolated from the gland, it was recorded as "Isolate 2". Coagulase negative staphylococci were the most prevalent isolate (13.5%), followed by *S. uberis* (2.8%). Other bacterial isolates were infrequent and grouped together as "other"; these were *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). Quarter IMI pre-calving due to major pathogens occurred at lower prevalence than from minor pathogens (difference = -10.6%, CI for difference = -12.2% to -9.1%).

Dual infections in quarters pre-calving were uncommon- less than 2% of quarters had 2 different isolates identified in the same quarter.

Table 2. Count and percentage ( ) of results of bacteriological sampling from heifer quarters and clinical mastitis quarters over prepartum and peripartum period in 708 pasture-grazed dairy heifers.

Days relative to calving	Prevalence of IMI -9 to -127 days pre-calving				Prevalence of IMI 0-5 days post-calving				Results from cases of clinical mastitis 0-14 days post-calving			
	Isolate 1		Isolate 2		Isolate 1		Isolate 2		Isolate 1		Isolate 2	
Quarters (n)	2832				2664				195			
CNS[1]	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)			--	--		
Corynebacterium spp.	7	(0.2)	1	(0.0)	2	(0.0)						
Eschericia coli	7	(0.2)	1	(0.0)	14	(0.5)	3	(0.1)	7	(3.6)	2	(1.0)
Staphylococcus aureus	12	(0.4)			16	(0.6)			5	(2.6)		
Streptococcus dysgalactiae	2	(0.1)			10	(0.4)			6	(3.1)		
Streptococcus agalactiae	1	(0.0)			--	--			--	--		
Streptococcus uberis	78	(2.8)			267	(10.0)			125	(64.4)		
Other	14	(0.5)			2	(0.1)			0	0		
No growth	2208	(78.0)			2075	(77.9)			36	(18.6)		
No sample	47	(1.7)			8	(0.3)			1	(0.5)		
Major pathogen	107	(3.9)			307	(11.6)			143	(73.3)		
Minor pathogen	395	(14.6)			262	(9.9)			15	(7.7)		
All pathogens	502	(18.5)			569	(21.5)			158	(81.0)		

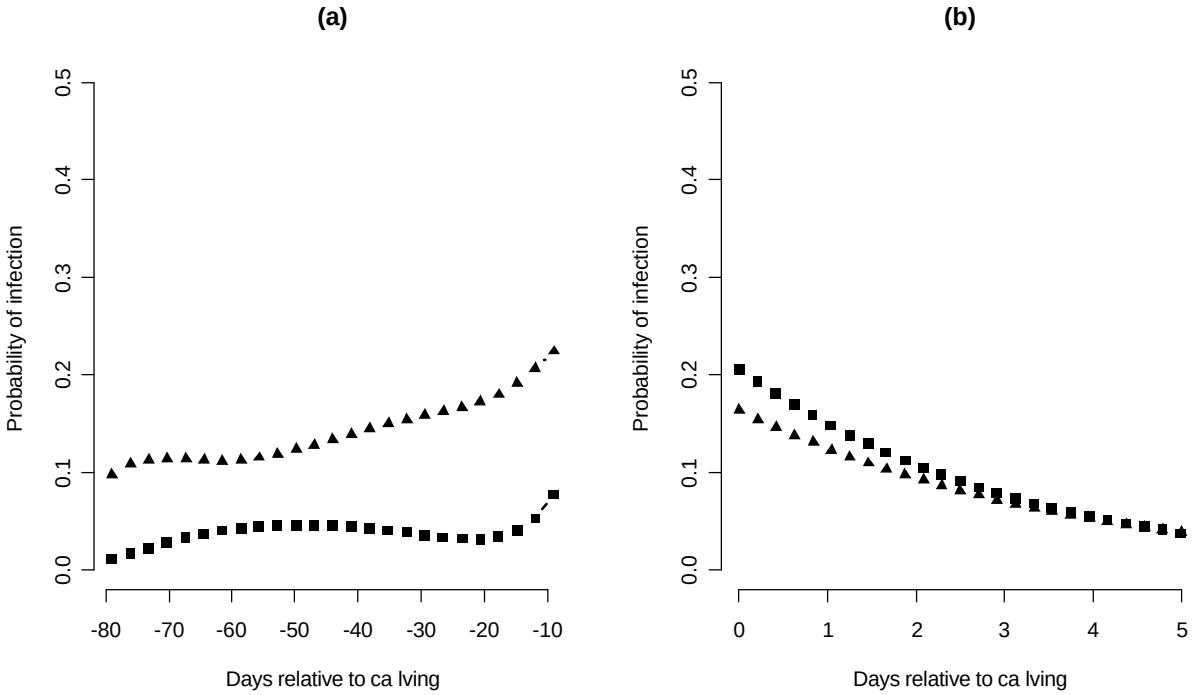
[1] Coagulase negative *Staphylococcus* species

The quarter-level prevalence of IMI increased from 18.5% pre-calving to 21.5% post-calving. For quarters with paired samples pre and post-calving, absolute prevalence increased by 2.9% (CI for difference = 1.1% to 4.7%). This change was due to a significant increase in major pathogen prevalence (particularly *S. uberis*) from 3.9% to 11.6% of quarters (for paired individual quarters difference = 7.7%, CI for difference = 6.4% to 9.0%).

Minor pathogen prevalence (mainly CNS) declined from 14.6% pre- calving to 9.9 % post-calving (for paired individual quarters difference = -4.7%, CI for difference = -3.2% to -6.3%). The prevalence of major pathogens was significantly higher than that of minor pathogens (difference = 1.7%, CI for difference = 0% to 3.4%) post-calving. Other pathogens were infrequently isolated, and dual isolates were recovered from only 2% of quarters involving principally minor pathogens isolated in conjunction with *S. uberis* or *S. dysgalactiae*.

Major pathogen prevalence remained low and relatively constant over the pre-calving sampling period, with a possible increase approaching day of calving (Figure 1a, b). However, the number of samples taken with 3 weeks of calving date was low and valid inferences could not be made. Minor pathogen prevalence was consistently higher than major pathogen prevalence pre-calving, and increased approached calving. Following calving, both major and minor pathogen prevalence declined rapidly after day of calving to less than 5% by the 5th day following calving.

Figure 1. Smoothed logistic regression of probability (prevalence) of quarter intramammary infection (IMI) by day relative to calving for major (• • •) and minor (• • •) pathogens pre- (a) and post-calving (b) in pasture-grazed dairy heifers.



Clinical mastitis was diagnosed in 195 quarters from 2784 quarters at risk within 14 days 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*, *S. dysgalactiae* and *S. aureus* (3-4% each). No bacterial isolates were recovered from 18% of samples submitted for culture from cows diagnosed with clinical mastitis, and no samples were defined as contaminated. Dual isolates from the same quarter with CM were diagnosed in only 8 (4%) cases, and in these CNS or *E. coli* were isolated in conjunction with *S. uberis*. Of the 569 quarters with any pathogen sampled within 5 days following calving, 96 were diagnosed with CM at the same time (16.9%, CI = 14.0% to 20.2%).

Bacteriological isolates from the same quarters were compared over time after data was coded according to the definitions in Table 1. Overall, 69.9% of quarters (CI = 68.1% to 71.7%) had samples both pre- and 0-5 days post-calving that were bacteriological negative. Of all pathogens isolated pre-calving, 35.8% (CI = 31.6% to 40.2%) had the same bacteria isolated post-calving. For minor pathogens, 31.5% of isolates (CI = 27.0% to 36.4%), and for major pathogens 51.5% (CI = 41.9% to 61%) of isolates were recovered a second time within 5 days of calving. Of all pathogens isolated pre-calving, 64.2% (CI = 59.8% to 68.4%) self-eliminated by the time of post-calving sampling. For minor pathogens, 68.5% of isolates (CI = 63.6% to 73.0%), and for major pathogens 48.5% of isolates (CI = 39.0% to 58.1%) self-eliminated over the pre- to post-calving sampling time period. A new bacterial isolate was recorded in 14.9% (CI = 13.6% to 16.3%) of all quarters over the pre- to post-calving interval. New IMI occurred in 14.7% (CI = 13.3% to 16.3%), 18.3% (CI = 14.7% to 22.6%) and 5.9% (CI = 2.8% to 12.4%) of initially bacteriological negative, minor pathogen and major pathogen positive quarters, respectively. New *S. uberis* infections occurred in 7.0% (CI = 6.0% to 8.2%) and 19.4% (CI = 15.7% to 23.7%) of previously IMI negative and minor pathogen positive quarters, respectively. Forty six of 48 quarters (96%) diagnosed with CM within 14 days following calving had the same isolate diagnosed at a prior sampling within 5 days

following calving. Four hundred and seventy-three quarters had isolates of any pathogen category isolated post-calving that were subclinical at the time of sampling, and of these 48 (10.1%, CI = 7.7% to 13.2%) were subsequently diagnosed as clinical cases within 14 days following calving.

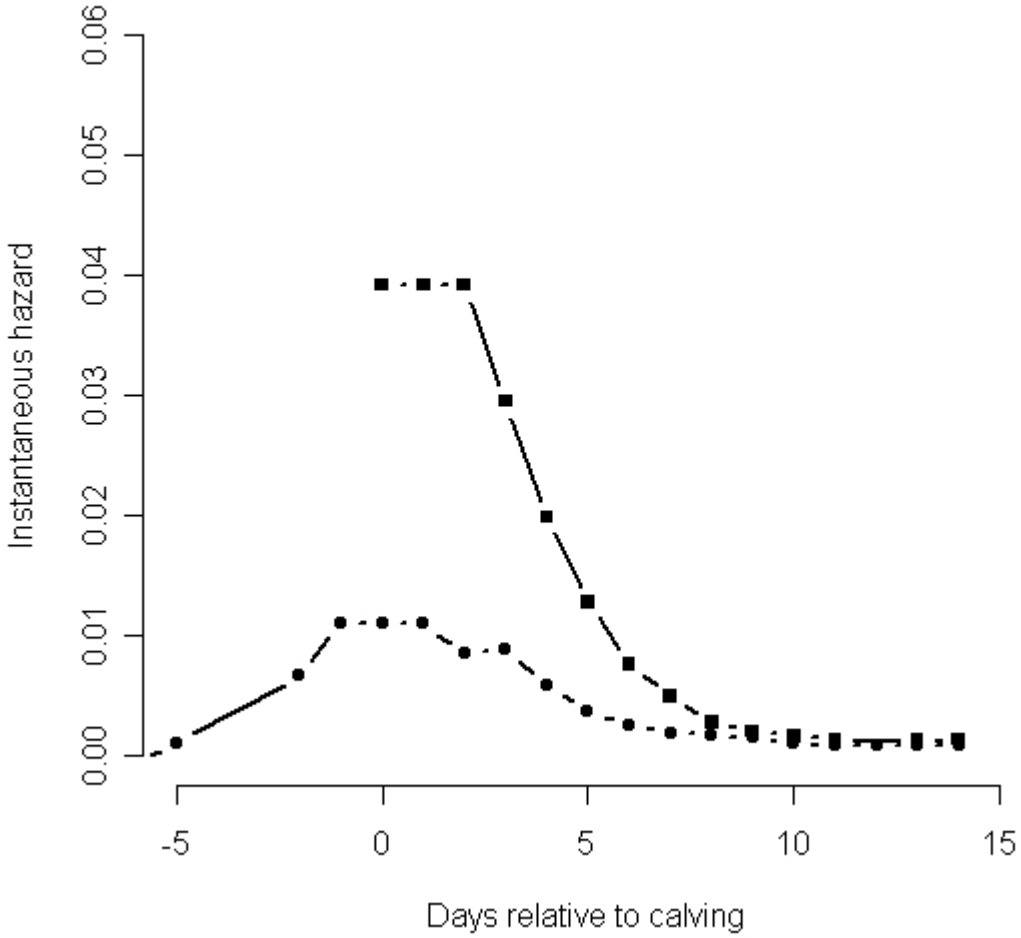
Pre-calving, 16.9% of front quarters were diagnosed with IMI, compared with 20.0% of rear quarters (difference=3.1%, CI of difference=0.2% to 6%). This was due to a higher proportion of major pathogen IMI in rear versus front quarters (4.7% vs. 3.1%, difference=1.6%, CI for difference=0.2% to 3.1%), but not of minor pathogens (15.3% vs. 13.8%, difference=1.5%, CI for difference=-1.2% to 4.1%). Post-calving IMI with any pathogen were more prevalent in rear quarters (26.9% vs. 16.1%, difference=10.8%, CI of difference=7.7% to 13.9%). This was due to higher prevalence of major pathogens in rear quarters (17.3% vs. 5.9%, difference=11.4%, CI of difference=9.0% to 13.8%), but not of minor pathogens (9.6 vs. 10.2%, difference=-0.6%, CI of difference=-2.9% to 1.7%). Clinical mastitis was more frequently diagnosed in rear compared to front quarters (cumulative incidence=0.102 vs. 0.038, difference 0.064, CI of difference=0.045 to 0.083).

Heifer-level results

Prior to calving, 268 of 708 heifers (prevalence = 37.8%, CI= 34.4% to 41.5%) had one or more quarters infected with any pathogen. Of these IMI, 182 (67.9%, CI = 62.1% to 73.2%) were due to minor pathogens. Post-calving, approximately one half (prevalence = 48.6%, CI=44.7% to 52.3%) of heifers had one or more quarters infected with any pathogen, a significant increase in prevalence over that pre-calving (difference=10.8%, CI of difference 6.2% to 15.4%). Minor pathogen prevalence decreased 10.1% (CI for difference = -6.0% to -14.2) from 25.6% to 15.5%, and there was a concurrent increase in prevalence of heifers with a major pathogen post-calving (pre-calving = 12.2%, post-calving=33.1%, difference=20.9%, CI of difference = 17.1 to 24.7%).

Clinical mastitis was diagnosed in 163 of 696 heifers at risk (cumulative incidence = 0.234, CI = 0.204 to 0.267) within 14 days following calving, and of these only 21 (3%) had diagnoses of CM in >1 quarters at the same time. The daily hazard or risk of diagnosis of CM in heifers was compared with that of all other age group cows using an instantaneous hazard plot (Figure 2). Heifers had a significantly higher daily hazard of CM in the first 14 days of lactation (hazard rate ratio from regression model=2.98, CI=2.56 to 3.47), than greater parity cows, and from inspection of the plot this occurs in the first week of lactation.

Figure 2. Smoothed instantaneous hazard (daily risk) of clinical mastitis in pasture-grazed dairy heifers (• ••) or in cows > 2 yrs of age (• ••) relative to individual calving date.



Pre-calving, IMI were isolated from 1, 2, 3 and 4 glands in 126(18%), 75(11%), 42(6%) and 25(4%) heifers, respectively. Post-calving, 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. One hundred heifers had both samples for clinical mastitis and routine post-calving sampling taken at the same visit. From the 83 heifers with 1 quarter diagnosed with CM, 41 (50%) had 2 or more additional quarters with any pathogen IMI, and of the 15 heifers diagnosed with CM in 2 quarters, 5 (38%) had 3 or more quarters with additional IMI.

Herd-level results

Wide variation existed between herds for all the outcome measures. Pre-calving IMI prevalence of heifers with any pathogen isolated from one or more quarters ranged between 18% and 66%, with that for major pathogens between 0% and 22%, and minor pathogens between 11% and 55%. Within-herd prevalence of heifers with any pathogen isolated from one or more quarters post-calving ranged between 10% and 80%, the range for major pathogens was 8% and 61%, and that for minor pathogens was 0% to 36%. The herd-level cumulative incidence of CM within 14 days following calving ranged between 7% and 48%, almost all of which was due to heifers with one or more quarters with clinical mastitis due to major pathogens (herd level prevalence range 3% to 44%).

Discussion

The pattern of prevalence and bacterial species involved in peripartum IMI in pasture-grazed dairy heifers in this study had some similarities, but also important differences from that reported in other countries and under different management systems. In common with findings from other studies, CNS prevalence was highest (13.5%) pre-calving, and declined as lactation commenced (9.7%). Reported quarter prevalence estimates for CNS were 16% to 14% to 6% (Oliver and Sordillo, 1988); 27% to17.8% to 12.1% (Aarestrup and Jensen, 1997) for 1-2 weeks pre-calving, calving, and 1-2 weeks post-calving, respectively. However, in this study, IMI prior to calving due to *S. aureus* was infrequent (0.4% of quarters), in contrast to the 1.2% found by Oliver and Mitchell (1983) and 14.9% by Trinidad et al. (1990), but similar to the 0.5% reported by Aarestrup and Jensen (1997). Possible reasons for this include the low population of insect vectors such as flies in the Waikato region of N.Z to transmit infection (Fox et al., 1995) and differences in housing and cow-heifer contact patterns . The prevalence of IMI for all pathogens pre-calviing in this study of 18.5% of quarters is lower than that of other previously cited authors (Oliver and Mitchell, 1983; Trinidad et al., 1990; Fox et al., 1995). Another feature of results from this study was the low prevalence of coliform and mixed IMI compared to other studies. Less than 0.5% IMI both pre- and post-calving in this study were due to Gram-negative bacteria, compared to 4.8% found by Oliver and Mitchell (1983) and 3% by Oliver and Sordillo (1988), and their prevalence estimates decreased only slowly post-calving. Prevalence estimates by Aarestrup and Jensen (1997) for coliform bacteria, however, were less than 1% throughout the peripartum period, in common with findings in this study. Intramammary infection with coliform bacteria is a recognized problem in dairy cattle managed under confinement systems due to fecal contamination of teat ends, but is apparently not a major problem under pasture-grazing systems in New Zealand (McDougall, 1998).

An important finding of this study is that *S. uberis* is by far the most common major pathogen causing IMI in both pre- and postpartum dairy heifers in New Zealand. *Streptococcus uberis* was isolated from 72% of major pre-calving and 87% of major post-calving infections. Pre-calving *S. uberis* prevalence of 2.8% of quarters in this study was slightly lower than the 4.8% found by Oliver and Mitchell (1983) and 3.4% by Oliver and Sordillo (1988), and similar to the 2% found by Aarestrup and Jensen (1997). However, post-calving *S. uberis* prevalence increased fourfold in this study to 10%, compared to reports of 7% (Oliver and Mitchell, 1983), 8% (Oliver and Sordillo, 1988), and 2% (Aarestrup and Jensen, 1997). Thus, the increase in *S. uberis* prevalence in this study was 2 or more times higher than in the other 3 studies. Reasons for the high relative importance of *S. uberis* in this compared to other studies are not known, but these findings are consistent in terms of relative importance, but of a higher magnitude, to that reported in post-partum heifers by Pankey et al. (1996) in a similar population. *Streptococcus uberis* is also the most common isolate from IMI and clinical mastitis in all parity cows early postpartum in NZ dairy systems (McDougall, 1998; McDougall et al., 2004), suggesting a high level of exposure to it in all parity groups under the grazing systems practiced in NZ. High densities of *S. uberis* have recently been demonstrated on the access tracks to the dairy parlour in NZ (Lopez-Benavides et al., 2005), which would provide such an exposure.

Comparing quarter infection status over time allowed description of the time period of highest infection rate. Approximately 80% of new major IMI's occurred in the last 2-3 weeks of gestation. From a plot of IMI prevalence by time relative to day of calving, major IMI prevalence did not appear to differ over the period -80 to -14 days, increased approaching calving, but then declined rapidly immediately post-calving. Therefore the majority of new *S. uberis* infections are occurring in the final 2 weeks of gestation in pasture-grazed dairy heifers. This is not likely to be an effect of calendar date as the calving dates varied across a period of July to September. Thus the increase in

incidence of new infection appears to be related to approaching calving, not some climatic effect through this period of time. Only a small proportion (10%) of quarters with subclinical IMI within 5 days following calving, were subsequently diagnosed with CM. This supports the finding from this study of a rapid decline in major pathogen IMI prevalence immediately post-calving. A proportion of these subclinical IMI may have persisted into lactation, but were not measured directly in this study.

The cumulative incidence of peripartum CM in this study also differed from the few described in other countries and management systems. No pre-calving CM was diagnosed in this study, unlike that of Trinidad et al (1990) in which there was a high quarter prevalence of CNS (6.6%) and *S. aureus* (3.3%) CM. This may be explained by the fact that pasture-grazed heifers are seldom closely examined prior to calving and their subsequent first milking (usually within 24 hours of calving). Cumulative incidence for CM of 7% of quarters and 23% of heifers within 14 days following calving in this study was higher than the 12% of heifers reported by Barnouin and Chassagne (2001) in the same time period, and the 8.1% of heifers reported by Pankey et al (1996) within 5 days following calving in similar herds to those in this study. 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), rarely causing clinical signs and responsible for only moderate increases in ISCC, they should not be disregarded as potential causes of CM in peripartum heifers. The finding in this study of a higher hazard of CM in heifers in the early post-partum period compared to higher greater parity cows is supported by that of Barkema et al. (1998). This difference between parity groups suggests different risk factors for CM operate for first versus greater parity cows and justifies consideration of mastitis in this age group as separate from others. The high incidence of CM in heifers found in this study is likely to impose significant costs on dairy producers, and warrants implementation of specific mastitis control programs for this parity group.

The distribution of IMI and CM between front and rear quarters differed, and this has implications for their diagnosis and aetiology. In this study, post-calving IMI due to major pathogens and CM were approximately 3 times more likely in rear compared to front quarters. Examination of all quarters and their secretion is important for diagnosing IMI and CM, but in herds where resources are limited, concentrating efforts on rear quarters of heifers in early lactation will enable diagnosis of most infections and clinical disease. Because minor pathogens (principally CNS) were isolated in equal proportions between front and rear quarters, support is given to the hypothesis that these are skin opportunist pathogens (Harmon and Langlois, 1989) present in similar concentrations on all teats and invading glands with non-functional teat plugs. In contrast, major environmental pathogens (principally *S. uberis*) preferentially infect hind quarters of heifers, which may be because transmission to these quarters is higher due to increased exposure via fecal or mud contamination or closer proximity to the ground; and probability of teat canal penetration increased; or a combination of both factors.

Isolation of *S. aureus* from quarter secretions from several weeks prior to calving in pasture-grazed dairy heifers shows they are a potential source of infection for this pathogen to other cows in the herd. *S. aureus* is usually considered to be a contagious pathogen spread between cows in lactation by the milking process, but this classification does not fit the pattern of infection of heifers pre-calving in this study. Other authors have drawn attention to this risk of introduction of this contagious pathogen (Boddie et al., 1987) and this possibility should be considered prior to introduction of purchased or self-sourced replacements to a herd with control programs specifically against this pathogen. Elimination of this intramammary pathogen may not be achieved without identification, isolation and treatment of post-partum heifers with this infection.

Knowledge of the prevalence of IMI at the heifer level is needed to assess preventive programs at this stage, as interventions are usually applied at the animal level. Few authors report IMI prevalence at the heifer level. Data from this study show that 38% of pasture-grazed heifers had one or more quarters with IMI in the pre-calving sampling period is much lower than the 97% found by Trinidad et al (1990), and presumably would be much lower than that reported by Oliver and Mitchell (1983) and Fox et al. (1995) who provided only quarter-level prevalence data. Although caution must be taken in making comparisons between bacteriological results from different studies due to differing methodologies, from the limited data available it would appear that NZ pasture-grazed dairy heifers have relatively low prevalence of IMI pre-calving compared to heifers under other management systems. However, prevalence of IMI pre-calving of 38% of heifers is still high and suggests significant exposure to pathogens prior to milking and a need for specific heifer control programs. The importance of pre-calving bacteriological status was demonstrated by the finding that the incidence rate ratio of new *S. uberis* IMI was significantly higher in quarters with minor pathogens compared to quarters with no pathogens isolated.

Herds varied widely in prevalence of IMI and incidence of CM, despite all using similar pasture-grazing management systems. This variation suggests that there is considerable scope for herd-level interventions or preventive strategies to reduce the heifer mastitis. New studies are required to understand herd-level risk factors for heifer IMI and CM under pasture-based systems.

Current mastitis control programs are not specifically designed for heifer peripartum mastitis, and are unlikely to be successful because the environment 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 infection rates due to *S. uberis* immediately before calving are required to reduce the incidence of CM in pasture-grazed dairy heifers. Although effective control of environmental mastitis apart from the use of non-lactating cow therapy has not been consistently reported in scientific literature, because of the preponderance of one bacterial species (*S. uberis*), targeted effective preventive measures against this one organism are likely to have a large population impact on CM in dairy heifers grazed on pasture. Further, this study has shown that there is wide variation in the within-herd proportions of heifers with IMI and CM, suggesting that herd level management options already exist to contribute towards control of heifer peripartum mastitis.

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