Prevalence of and Risk-Factors for Intramammary Infection in Pasture-Grazed Dairy Heifers in Late Gestation

Chris Compton, Scott McDougall and Fiona Anniss
Animal Health Centre, PO Box 21, Morrinsville, New Zealand

Introduction

Mastitis is a common infectious disease in dairy cattle, and is among the most economically damaging animal health problem producers face. Mastitis in dairy heifers is recognised as a distinct problem from that in older cows because of the different pattern of disease (Barkema, Schukken et al. 1999; Valde, Lawson et al. 2004) and unique factors that determine its occurrence (Waage, Sviland et al. 1998; Waage, Odegaard et al. 2001). Data from New Zealand (Compton, unpublished) shows that heifers have amongst the highest age-specific incidence of clinical mastitis (CM) (up to 5%) and that the largest majority of cases are diagnosed in the first 2 to 3 weeks of lactation. Other NZ data (Compton, unpublished) shows that intramammary infection (IMI) between 12 and 2 weeks pre-calving is common but is not as high as at calving. But no data is available to define when most new infections occur pre-calving or what factors may be associated with them. Knowledge of both these factors is important for developing control programmes for heifer mastitis.

Several risk factors for pre-calving IMI have been reported, including seasonal and regional effects (Fox, Chester et al. 1995; Waage, Sviland et al. 1998). Several authors have also reported increasing prevalence of IMI with increasing days of gestation (Oliver and Sordillo 1988; Aarestrup and Jensen 1997). However, there are no studies reported investigating risk factors for IMI operating at the quarter-level in late gestation and conducted under pasture-grazing systems as in New Zealand.

The route of infection for mastitis in dairy cattle is almost exclusively through the teat canal, and hence several workers have investigated associations between teat canal characteristics and IMI. Formation of a keratin mass (“teat plug”) in the teat canal over the non-lactating period in multiparous cows has been found to be significantly protective against new IMI (Williamson, Woolford et al. 1995; Lacy-Hulbert, Williamson et al. 1999; Dingwell, Leslie et al. 2004). However, there are no published reports of on the prevalence, characteristics and role of teat plugs in dairy heifers prior to their first calving.

Descriptions of methods to weigh teat plug material and determine the presence or absence of teat plugs in non-lactating cows have been described. An invasive method to remove teat canal keratin material using the eye of a darning needle for weighing provides objective and repeatable data (Bright, Bitman et al. 1990). Another subjective and non-invasive method of assessing functional teat closure (FTC) uses light digital pressure to determine whether mammary secretion in the teat sinus is capable of passing through the teat canal to be visualised as secretion leaking from the orifice (Williamson, Woolford et al. 1995). However no descriptions exist of the use of these methods in heifers prior to their first calving and associations between the results of these tests and IMI.

The main aim of this study was to estimate the prevalence of IMI in heifer quarters in the last two weeks of gestation and thereby define the time period relative to day of calving of greatest risk of...
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new IMI. Secondary aims were to investigate associations between weight of teat canal keratin and both intramammary infection status and FTC, and to describe and investigate risk factors operating at the quarter level for IMI in heifers in late gestation.

Materials and Methods

Heifers (n = 247) from 4 commercial dairy herds in the Waikato synchronised to calve around two dates in each herd in July and August 2006 (range of expected average calving dates 2nd July to 8th August 2006) were enrolled in this study. The number of heifers totalled 177 in the first round of calving and 70 in the second round. Because not all heifers conceived to synchronised matings, each herd had non-enrolled heifers also calving to natural matings, but these were not sampled. Heifers were categorised for breed as Friesian (> 11/16ths Friesian) (n = 122), Jersey (> 11/16ths Jersey) (n = 83) and other (mainly crossbred) (n = 42). Herds were considered eligible for the study if they were clients of Animal Health Centre (Waikato Inc.), had synchronised artificial breeding for their yearling replacements in October 2005, had undertaken pregnancy testing of heifers early enough to confirm date of conception, and were willing to comply with the requirements of the study. Individual heifer data on breed and identification were obtained electronically from Livestock Improvement Corporation (Hamilton, NZ) prior to enrolment. Power analysis was undertaken to estimate the sample size required to estimate prevalence of between 5% and 20% (expected range in prevalence of all IMI at 14 and 2 days prior to calving, respectively) with precision of +/- 2.5% and 10% respectively, resulting in quarter sample numbers between 92 and 61, respectively. Prior approval for conducting this study was gained from the AgResearch Ruakura Animal Ethics Committee.

Prior to enrolment, quarters of each heifer were randomly assigned for sampling of mammary secretion to be cultured for bacteriology. Each quarter was sampled on one of four dates prior to calving. Dates for successive samplings were 8, 6, 4 and 2 days prior to the mean expected date of calving for heifers in the herd in each of two rounds of synchronised calving. Because the exact date of calving could not be predicted, and the average gestation was 282 days with a standard deviation of 4.5 days, it was expected that approximately half the heifers would calve before all 4 quarters were sampled. The number of days between sampling and calving was expected to vary between 17 (8 days prior to expected calving date plus two standard deviations) and zero (as quarters were not sampled after calving). Thus each quarter was sampled once only before calving, and the number of quarter samples per heifer varied from 1 to 4.

Single or duplicate (when secretion volume was adequate) milk-samples for bacteriology were taken following scrubbing of the teat ends in cotton wool swabs soaked in 70% alcohol. Samples were transported chilled to the laboratory for processing within 2 hours of collection. Because the mammary secretion sampling procedure was expected to increase the probability of a quarter becoming subsequently infected following opening of the teat canal, approximately half the quarters were randomly assigned to be treated with Teatseal® (Pfizer Animal Health, NZ Ltd) according to the manufacturer’s directions immediately after sampling to reduce the risk of new infections. The other quarters were left untreated to be sampled at the first milking for another study to be reported elsewhere. After mammary secretion sampling or use of Teatseal® (Pfizer Animal Health, NZ Ltd) the individual teat that had been sampled or treated was sprayed with a teat sanitiser solution containing 0.2% available iodine. Microbiological procedures, diagnosis of IMI, and categorisation of results were undertaken using standard methodology (Hogan, Gonzalez et al. 1999).
Repeated measurements of putative risk factors at the quarter level for all quarters were taken at the same mammary secretion sampling visits until calving for each heifer. Quarter-level scores were recorded for quarter oedema (on a 1 to 3 scale with 1 = no oedema, 2 = mild oedema, 3 = severe oedema), quarter hygiene (modified from that of Schreiner and Ruegg (2003) with 1 = clean, 2 = slightly dirty, 3 = mostly covered in dirt, 4 = completely covered in dirt), quarter milk leakage (on a 1 to 3 scale with 1 = no leakage, 2 = traces of milk observed on teat apex, 3 = milk dripping or flowing from gland) and absence or presence of FTC using the method reported by Williamson et al. (1995) (on a 0 or 1 scale, with 0 = no milk leakage observed with light digital pressure to the teat cistern, and 1 = some milk leakage observed. Height of apex of teats above the floor of the milking parlour was measured using a flexible tape measure once only for each teat on the day the quarter had a mammary secretion sample taken for bacteriology.

In the course of the field work, technicians observed that some teat ends had a visible “button” of dry material like a membrane covering the orifice that was removed with scrubbing with cotton wool swabs soaked in alcohol prior to mammary secretion sampling. The absence or presence of these “teat buttons” was therefore recorded in heifers in the second round of synchronised calvings (n = 59 heifers meeting the inclusion criteria), and its association with IMI was investigated.

In addition to the above measurements and samples, teat canal keratin samples were also collected. In a random sample of 20 heifers in 3 of the 4 herds, at the first visit at 8 days prior to average calving date, teat canal keratin was removed for weighing and culture using the darning needle method (Bright, Bitman et al. 1990) from the same quarter that had been pre-assigned for bacteriological sampling. The sample was weighed within 2 hours of collection and directly plated onto culture medium for bacteriological testing.

Bacteriological results were categorized as either major or minor pathogens. Bacterial species classified as major pathogens were Enterococcus spp., Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and S. uberis. Minor pathogens were CNS, Corynebacterium spp., undifferentiated gram positive rods and yeast. When a quarter had both a major and a minor pathogen isolated at the same time, the quarter was given the ‘major pathogen’ status. In calculating proportions, samples that were either contaminated or not collected were not counted in the denominator.

Farmer-diagnosed cases of CM (presence of clots or changes in composition of milk, or presence of swelling or heat in a quarter) were recorded in both enrolled and non-enrolled heifers to evaluate whether sampling procedures had affected the incidence of CM. However, the cumulative incidence of CM post-calving was not a major response of interest because the pre-calving sampling methods were expected to bias the association between risk factors and outcome. Therefore, associations between risk factors and CM were not evaluated.

Estimates of quarter prevalence of IMI for quintiles of days prior to individual calving dates were calculated for pathogen categories (major or minor) and 95% confidence intervals for differences in proportions calculated using Newcombe’s method 10 (Newcombe 1998). Smoothened logistic regression methods implemented in the R (R Development Core Team 2006) add-in package “sm” (Bowman, Azzalini et al. 2005) were used to plot the probability of IMI (equivalent to IMI prevalence) and prevalence of putative risk factors for IMI by day prior to individual calving date. The Chi-square test for trend in proportions was used to test for differences in proportions over time. The association between weight of teat canal keratin and IMI status was investigated using one-way analysis of variance.
Associations were investigated between putative risk factors as the outcome variable and day of measurement prior to calving and quarter position (left fore, left rear, right fore, right rear). Ordinal risk factor measures were dichotomised and associations tested using generalised estimating equations with logistic regression for repeated measurements with exchangeable correlation structure between quarters of a heifer in the “geepack” package in R (Yan and Fine 2004). Associations were also investigated between quarter IMI status and the highest level of putative risk factor for ordinal variables recorded prior to or on the day of quarter mammary secretion sampling and the day of sampling prior to calving. For the dichotomous variables FTC and teat button, the reference level was presence of the characteristic (score = 2) at that and all of any previous records. Firstly, crude associations were estimated for categorical variables, and then adjusted associations estimated using generalised estimating equations logistic regression models as described before. Herd, quarter position and day prior to calving were included in the model as fixed effects. The interpretation of output from logistic regression models is in terms of odds ratios, which is not an approximation of the more readily-interpreted incidence risk when the outcome is not rare. Hence, the results of multivariable logistic regression models were converted from odds ratios to incidence risk ratios (RR) with 95% confidence intervals using the method of Beaudeau and Fourichon (1998).

Data was recorded into a Microsoft Access database, and statistical analysis carried out using R (R Development Core Team 2006). Statistical significance was declared for tests with Type I probability values ≤ 0.05.

**Results**

The calving dates for heifers were a median of 1 day after the expected average calving date (range -26 to 24 days, respectively). Data from 21 (8.5% of enrolled heifers) was excluded from further analysis because they calved before the first planned visit (n = 9), calved > 14 days after their last sampling (n = 8) or failed to calve (n = 4). One heifer was not presented for sampling on the first visit 8 days prior to its predicted calving, but data from subsequent visits were included in analysis. Samples and measurements were collected from 225, 215, 202 and 171 heifers at planned visits on 8, 6, 4 and 2 days, respectively, prior to expected group average calving dates. Mammary gland secretion samples were collected between 9 and 0 days before the day of calving of individual heifers (mean = 7.3 d, SD = 4.1 d). Samples were collected from 205, 199, 201 and 206 left fore (LF), left rear (LR), right fore (RF) and right rear (RR) quarters, respectively.

Of 811 quarters sampled pre-calving, 532 (65.6%) yielded no growth, 144 (17.8%) CNS, 58 (7.2%) S. uberis, and no mammary secretion sample could be collected from 32 (3.9%) of quarters. Other mammary pathogens isolated were E. coli, S. dysgalactiae and S. aureus but these totalled less than 2% of samples. Two bacterial species were isolated from only 29 samples, and of these all except 1 (an E. coli isolate) were CNS. After excluding contaminated (n = 5) samples and quarters without sample results, minor pathogens (almost all CNS) were isolated from 150 (19.4%) quarters, and major pathogens (almost all S. uberis) were isolated from 75 (9.7%) of quarters.

Prevalence of each pathogen type by day pre-calving is shown in Figure 1. Within each pathogen type, prevalence did not vary significantly over time (P = 0.35 and P = 0.28, for minor and major pathogens, respectively). Prevalence of minor pathogens and major pathogens did not differ significantly by teat position (P = 0.56 and P = 0.95, respectively). Differences in prevalence between pathogen types were significant (P ≤ 0.05) within periods -19 to 12, -12 to -8, and -8 to -4 days pre-calving, but not within periods -4 to -2 and -2 to 0 days pre-calving (P > 0.2).
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Figure 1. Prevalence of intramammary infection by pathogen type and day prior to individual calving

![Prevalence of intramammary infection by pathogen type and day prior to individual calving](image)

The cumulative incidence of clinical mastitis in quarters of enrolled heifers diagnosed by farmers up to 90 days post-calving was 6.8% compared to 6.7% in non-enrolled heifers (P-value for difference = 1). Ten quarters (27% of cases) were diagnosed with CM before the day of calving in enrolled heifers. The cumulative incidence of clinical mastitis in quarters that had been sampled was 6.3%, which was not different (P-value = 0.20) from quarters that had not been sampled (9.1%). The cumulative incidence of clinical mastitis cases in all heifers up to 90 days post-calving was 20%, and this did not differ between enrolled (19%) and non-enrolled (22%) heifers (P-value = 0.44).

Over all scoring events, the most common udder hygiene scores were 2 (31%) and 4 (30%) (Table 1). The change in average quarter hygiene score for different quarters as calving approached is shown in Figure 2. Quarter hygiene was more likely to be categorised as ≥ 2 score (dirty) than 1 (clean) as day of calving approached (P < 0.01), if the quarter was a rear quarter (P < 0.01), and if the teat height above the ground was lower (P < 0.01).

Figure 2. Average udder hygiene score by quarter position and day relative to calving

![Average udder hygiene score by quarter position and day relative to calving](image)
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Table 1. Descriptive statistics of categorical and continuous risk factors investigated for pre-calving intramammary infection in dairy heifers

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Score</th>
<th>Hygiene (%)</th>
<th>Oedema (%)</th>
<th>FTC (%)</th>
<th>Teat button (%)</th>
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<tr>
<td></td>
<td>1</td>
<td>540 (17)</td>
<td>1304 (40)</td>
<td>164 (5)</td>
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<td></td>
<td>2</td>
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<td>1311 (40)</td>
<td>3080 (95)</td>
<td>506 (58)</td>
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<td>725 (22)</td>
<td>629 (20)</td>
<td></td>
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<td></td>
<td>4</td>
<td>988 (30)</td>
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<td>0</td>
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Table 1 continued

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Score</th>
<th>Teat height</th>
<th>TC keratin wgt (mg)</th>
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<td></td>
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<td>51.0</td>
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<td>21</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0</td>
<td>19</td>
</tr>
</tbody>
</table>

1 For ordinal variables, 1 = absent and increasing score indicates increasing severity of condition; for dichotomous variables, 1 = absent, 2 = present
2 Functional teat closure
3 Dry membrane-like substance covering teat orifice assessed only in 2nd round of synchronised calvings
4 Height of teat apex above floor of milking area
5 Teat canal keratin

Quarter oedema scores 1 and 2 were equally prevalent (40% each), with the most severe oedema score (3) recorded on 20% of occasions. Average quarter oedema scores for both front and rear quarters relative to day prior of calving are shown in Figure 3. Quarter oedema scores ≥ 1 were significantly more common as calving approached (P < 0.01) and in rear versus front quarters (P < 0.01).

Figure 3. Average quarter oedema score by quarter position and day relative to calving
Only 5% of quarters were found to have open teat canals across all observations. The prevalence of quarters with open teat canals by both quarter position and day relative to calving is shown in Figure 4. Quarters were more likely to have open teat canals as calving approached (P < 0.01) and if they were rear quarters (P < 0.01).

Teat buttons were recorded on 58% of quarters scored in the second round of calving. Because the observation of these teat buttons was opportunistic, only a relatively small number of teats were scored for their presence (n = 876 scoring occasions). Teat buttons were less prevalent as calving approached (P < 0.01), but no pattern amongst quarters of different position was found (P > 0.6).

Average teat height above the floor of the milking area was 53.9 cm (SD = 4.0 cm). Teat height decreased as calving approached (P < 0.01) and was lower for front compared to rear quarters (P < 0.01). Quarter hygiene was negatively associated with teat height above the ground. For each 1 cm increase in teat height the percentage of quarters with hygiene score ≥ 2 increased from 9% to 94%.

Teat canals from 72 quarters were sampled for bacteriology and keratin removed for weighing. One sample was classified as contaminated and removed from further analysis. No pathogens were isolated from 46 samples (65%). Mammary pathogens were isolated from 25 teat canal keratin samples (35%), and these were predominantly minor pathogens (CNS, n = 20), with only 4 S. uberis and 1 S. dysgalactiae isolates. Teat canal keratin infection status was significantly associated with intramammary infection status (P < 0.01) for both pathogen categories. The same bacteriological result was obtained for both teat canal keratin and mammary secretion sample in 91% of cases. Teat canal keratin samples could not be recovered from 19 quarters. Average weight of recovered teat canal keratin was 22.3 mg (SD = 21.1 mg). The average weight of teat canal keratin in quarters with any IMI did not differ from those with no IMI (26.6 mg vs. 20.5 mg, P = 0.39), and neither did the weight of teat canal keratin differ between quarters with a major pathogen IMI compared with no major pathogen IMI (16.2 mg vs 23.1 mg, P = 0.85). There were insufficient numbers of quarters which had both teat canal keratin samples and open teat canals (n = 1) to assess any association between these two variables.
Milk leakage was recorded on only 6 quarter sampling occasions. Because of the rarity of this condition in this study, the records were not analysed. Teat buttons were only recorded in the second round of calvings, hence investigation of their association with IMI status could only be done using crude associations and a fraction of the full data set. The prevalence of IMI due to all pathogen types in quarters with teat buttons was 23% compared to 40% in quarters without teat buttons (RR = 0.56 CI = 0.37 to 0.86, P < 0.01). The prevalence of IMI due to major pathogen types was not significantly associated with presence of teat buttons; 9% in quarters with teat buttons compared to 16% in quarters without teat buttons (RR = 0.54 CI = 0.25 to 1.15, P = 0.15).

The results of multivariable analysis showed that quarters that always had FTC prior to or on the day of mammary secretion sampling were 0.47 times (95% CI = 0.43 to 0.51) less likely to have an IMI with any pathogen (P < 0.01) and 0.47 times (95% CI = 0.26 to 0.81) less likely to have an IMI with a major pathogen (P < 0.01). Risk of quarter IMI with a major pathogen was also 1.8 times (95% CI = 1.0 to 3.2) times greater when that quarter had udder oedema score ≥ 2 (moderate or severe oedema). Teat position, udder hygiene, and day of sampling relative to calving were not significantly associated with IMI after adjustment for other variables in the final model. The relationship between prevalence of minor pathogen and major pathogen IMI in quarters grouped by FTC status and by day prior to calving is shown in Figures 5 a and b, respectively.

Figure 5. Prevalence of intramammary infection due to minor (a) and major (b) pathogens by status of teat canal closure status and days relative to individual calving

Discussion

The cumulative incidence of clinical mastitis in heifers in this study was not different from that found in other surveys. Neither was the incidence of clinical mastitis different between enrolled and non-enrolled heifers. In fact, the incidence was numerically lower in sampled heifers, possibly due to the protective effect of Teatseal® used post-sampling. Apparently the sampling procedure did not have any significant adverse effect on the heifers.

The prevalence of IMI pre-calving was higher than that previously found in a similar population of heifers (Compton, unpublished). In that study, between 9 and 127 days pre-calving, minor pathogen prevalence averaged 14.6% and major pathogens 3.9% of quarters, compared with
19.4% and 9.7%, respectively, in this study. In common with that data though, major pathogens other than \textit{S. uberis} and mixed infections were infrequent. This again emphasises the importance of control of \textit{S. uberis} to reduce heifer mastitis in NZ pasture-grazing systems.

An important finding of this study was that there was no significant change in IMI prevalence over the last 2 weeks of gestation. The incidence rate of new IMI was not directly tested in the conventional way by repeated sampling of the same gland over time because it was believed that this would itself affect the risk of new infections occurring due to opening of a previously closed teat canal facilitating bacterial invasion, thus biasing the results. (Such repeated sampling of quarters is appropriate for cows in lactation because sampling is not unlike the normal milking procedure). Prevalence of infection is determined by both the incidence of new infections and cure of existing infections. Because of the study design the rate of IMI self-cure could not be estimated in this study. However, the time periods over which sampling was undertaken were brief and most of the mammary pathogens isolated were capable of causing infections of at least several days duration, hence it is reasonable to believe that self-elimination was not common. Hence, no change in IMI prevalence implies that the incidence of new IMI in heifers in the last 14 days of gestation was negligible. Intramammary infections have been diagnosed in dairy heifers as young as the age of first breeding (Trinidad, Nickerson et al. 1990), and prevalence found to vary by region and season (Fox, Chester et al. 1995). Lack of control of insect vectors (mainly horn flies) (Nickerson, Owens et al. 1995) and intersuckling (Keil, Audige et al. 2000) have been suggested risk factors for IMI of calves and young heifers.

Estimates of prevalence of IMI for major and minor pathogens in a similar population at the first milking on the day of calving from a previous study (Compton, unpublished) were 20% and 17%, respectively. If that data was representative of pasture-grazed heifers in general and could be applied to this data set, then on the day of calving, new infections with minor pathogens were uncommon (17% prevalence on day of calving in previous study, 19% prevalence pre-calving in this study). However, under the same considerations the incidence of new major IMI appeared to be very high (20% prevalence on day of calving in previous study, 10% prevalence pre-calving in this study). Hence factors acting on the day of calving are likely to be critical for the establishment of new infections with major pathogens.

Data from this study does not show an important protective effect of weight of teat canal keratin against IMI because the weight of teat canal keratin was not associated with quarter IMI status. However, teat canal keratin may still have important protective roles not related just to its mass that can be recovered. Also, the same bacteriological result for the same quarter for both teat canal keratin and mammary secretion was found in 91% of cases, suggesting that the same bacteria could penetrate the teat canal and infect the mammary gland. Data from cows in the non-lactating state show that teat canal keratin provides an important protective effect by physically blocking the teat canal space and producing antibacterial factors (Hogan, Smith et al. 1988; Dingwell, Leslie et al. 2004). However, closure of the teat canal is also related to a balance of teat end tissue contractile mechanisms from smooth muscle and elastic tissue, and hydrostatic pressure from accumulating colostrum within the teat cistern (van der Merwe 1985). Together, these factors may be more important than teat canal keratin for teat-end defence against bacterial invasion in pre-partum heifers.

Functional closure of the teat canal as determined by the method described by Williamson (1995) was found to be strongly protective against new infection by both minor and major pathogens. The prevalence of FTC declined as date of calving approached. This data combined with the finding that teat canal keratin weight was not associated with IMI status, supports the view that lactogenesis and increased hydrostatic pressure overcoming contractile mechanisms in the teat
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end increase the risk of IMI. Other international studies have shown that milk leakage pre-calving in heifers may be increases the risk of mastitis both pre-calving and post-calving (Waage, Sviland et al. 1998; Waage, Odegaard et al. 2001). Milk leakage pre-calving in this study was rarely reported, however. This may be due to differences in breeds and nutrition between the farming systems whereby heifers with high genetic potential for milk yield and optimally fed for production in intensive systems produce store more milk pre-calving making milk leakage more common, compared to pasture-grazed heifers in NZ selected for higher percentage milk components.

Other known risk factors for IMI were found to be more prevalent as day of calving approached. Udder hygiene and udder oedema have been found both in NZ (Compton, unpublished) and internationally to increase the risk of IMI (Schreiner and Ruegg 2003) and CM (Slettbakk, Jorstad et al. 1995; Waage, Odegaard et al. 2001) post-calving. In the current study however, poor quarter hygiene did not increase the risk of IMI pre-calving. This may be because poor udder hygiene only increases the risk of IMI when there is the absence of FTC i.e. open teat canals are a necessary cause. The prevalence of FTC pre-calving in this study may have been too high to find an association between udder hygiene and IMI, even though other workers have found this association post-calving. Although it may seem self-evident that teat canals must be sufficiently “open” to permit entrance of pathogens leading to IMI, this study has shown that a simple cow-side test may be useful for assessing heifer risk for IMI. Udder oedema was found in this study to increase the risk of IMI pre-calving with major, but not minor pathogens. Earlier work (Compton, unpublished) in a similar population found udder oedema was a significant risk factor for CM post-calving but not for subclinical mastitis. The mechanism by which udder oedema influences whether an infection with a major pathogen establishes and becomes clinically apparent are unknown, but appear to operate pre-calving in addition to post-calving. These factors are likely to be related to udder immune system competency and possibly the flushing effect of the first few milkings. Demonstration from this data that new risk factors (open teat canal) for pre-calving IMI, and known risk factors (oedema, poor hygiene) for post-calving IMI and CM were more common in rear compared to front quarters may in part explain the finding of increased risk of IMI and CM in quarters of that position (Compton, unpublished).

Conclusion

Factors that operate on the day of calving are likely to have a major influence on the prevalence of IMI at that time. Data from this study suggests that although the prevalence of major pathogen IMI is high and relatively constant in the last 14 days of gestation, a high incidence rate of new infection is likely to occur on the day of calving. Quarters which were assessed to be open, even though milk leakage was not observed, were significantly more at risk of infection. Hence there is a need in developing heifer mastitis control programmes for increased understanding of factors associated with openness of teat canals, and management of the calving process and first few milkings.

Acknowledgements

We thank our team of technicians who sampled the heifers and recorded data on-farm- Fiona Anniss, Laura Haakma, Braydon Smith, Sally Hughes-Ross and Rhonda Cooper, and Maggie Collinson for specimen reception and data entry. We especially thank the farmers and their staff who provided their stock for the study and gave us access to their records. We finally thank Dairy Insight for funding the study as part of Project No. 20017 “Reducing Mastitis in Heifers” and co-funding from Sustainable Farming Fund (Grant No. 06/004).
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