Path analysis for evaluation of risk factors for dairy heifer mastitis in New Zealand

C.W.R. Compton[1], C. Heuer[2], K. Parker[1] and S. McDougall[1]

[1]Animal Health Centre, P.O Box 21, Morrinsville, New Zealand; [2]Epicentre, Massey University, Palmerston North, New Zealand.

Abstract

A longitudinal observation study was undertaken among 708 seasonal calving pasture-grazed dairy heifers in the Waikato region of New Zealand with the aim of determining risk factors for peripartum mastitis and to estimate their relative population impact to inform preventive measures. Mammary secretion, blood samples, and several measurements were taken before the start of the calving period, and within 5 days of calving of each heifer. Additionally, all cases of clinical mastitis (CM) within 14 days of each heifer calving were sampled for bacteriology. Multilevel logistic regression models in combination with path analysis were used to investigate postulated causal pathways between risk factors for CM and subclinical mastitis (SCM) post-calving. Significant risk factors for SCM were found to be pre-calving intramammary infection (IMI), low minimum teat height above the ground and poor udder hygiene post-calving. Significant risk factors for CM were pre-calving IMI, Friesian breed, low minimum teat height above the ground independent of breed, udder oedema, and low post-calving non-esterified fatty acid serum concentration. Possible causal pathways for SCM and CM are discussed, and preventive measures against both exposure and host factors recommended.

Introduction

Mastitis in dairy cows is common and is likely to impose significant economic costs on producers. National surveys of dairy herds show the incidence of clinical mastitis to be among the highest of reported diseases (Xu and Burton, 2003). A review of the economics of mastitis and it's control by Schepers and Dijkuizen (1991) estimated the costs of clinical mastitis (CM) to be in the range of U.S $100-300 dollars per cow per lactation averaged over the total herd. Total costs attributable to mastitis in dairy heifers specifically have not been published, but estimates of milk production losses following CM in heifers have ranged from 0 to less than 10% of the total yield of the first lactation (Myllys and Rautala, 1995; Hortet and Seegers, 1998).

Studies report different patterns of infection in heifers compared to older cows. Data from Barkema et al, (1998) emphasized the importance of peripartum mastitis in dairy heifers by showing that >30% of cases of CM in heifers were diagnosed in the first 14 days of lactation, compared to 13% in all other parity groups combined. A survey of bovine mastitis treatments in Nordic countries, Valde et al. (Valde et al., 2004) also found higher cumulative risks for parity 1 cows compared to parities 2 and 3 in the first 2-3 weeks of lactation.

Important risk factors for mastitis in dairy cows depend on the pathogens involved. For contagious pathogens, these include persistent intramammary infection (IMI) from the previous lactation, transmission of pathogens via milking cup liners due to inadequate machine function, poor milking technique, and inadequate teat sanitization and presence of teat lesions. Important risk factors for IMI due to environmental mastitis pathogens include failure to form an effective teat plug in the non-lactating period, presence of teat end lesions, increased exposure to contamination from bedding, wet udder milking preparation and milk leakage. However, the control of mastitis pathogens of environmental origin using the same measures has not been as successful (Bramley, 1984).

Knowledge that the pattern of CM and subclinical mastitis (SCM) in periparturient heifers differs from that in higher parity cows has prompted research into defining risk factors that determine

mastitis incidence in this parity group alone. Different management systems and physiological status related to age and lactation of heifers, compared to those of cows are likely to be important, and several risk factors for peripartum CM and IMI in heifers have been identified in mainly confinement management systems. However, the seasonal calving and pasture management systems commonly used in New Zealand may lead to different risk factors or of different relative importance to those from studies undertaken in confined non-pasture fed cattle.

Few preventive programs are available specifically aimed at controlling mastitis in peripartum dairy heifers. Effective fly control was recommended by Trinidad et al. (1990a) where these insects act a vectors of infection before calving. Separation of pre-weaned calves to prevent intersuckling and segregation of pregnant heifers from dry cows were suggested by (Shearer and Harmon, 1993) to reduce transmission of pathogens. The use of prepartum intramammary antibiotics to treat and prevent IMI in primigravid heifers has been reported by several authors (Trinidad et al., 1990b; Owens et al., 2001; Oliver et al., 2004) with high rates of efficacy reported, but problems exist with prevention of antibiotic residues entering human food supply.

Objective

The objective of this study was to determine risk factors for peripartum IMI and CM in pasture-grazed dairy heifers that operate at both the quarter and heifer level and describe them using path analysis methods. A secondary aim was to estimate the proportion of cases in the population attributable to the identified risk factors for subclinical and clinical mastitis, and thereby to determine factors for which effective control would provide the greatest population benefit.

Methods

This was a longitudinal observational study in a closed population, which started in June 2004. Heifers (n=708) were randomly selected from a convenience sample of 30 spring seasonal-calving herds (6-27 heifers per herd). Heifers were enrolled on one occasion approximately 4 weeks before the planned starting date of calving for the heifers in each herd. At enrolment, single samples of intramammary secretion from each quarter were aseptically collected for microbiological testing (Hogan et al., 1999), in addition to duplicate blood samples for frozen storage later analysis of beta-hydroxy butyrate (BOH) and non-esterified fatty acids (NEFA), and records made of degree of udder contamination using a hygiene scoring system (Schreiner and Ruegg, 2002): 1) complete or almost complete freedom from dirt; 2) slightly dirty; 3) mostly covered in dirt; 4) completely covered in dirt; body condition score (Macdonald and Roche, 2004) recorded on a 1-10 ordinal scale with half score increments; and length of the tail of each heifer was recorded as: 1, docked short (<20 cm in total length); 2, docked medium length (20-40 cm total length); 3, natural length but with the twitch trimmed; 4, natural length and untrimmed.

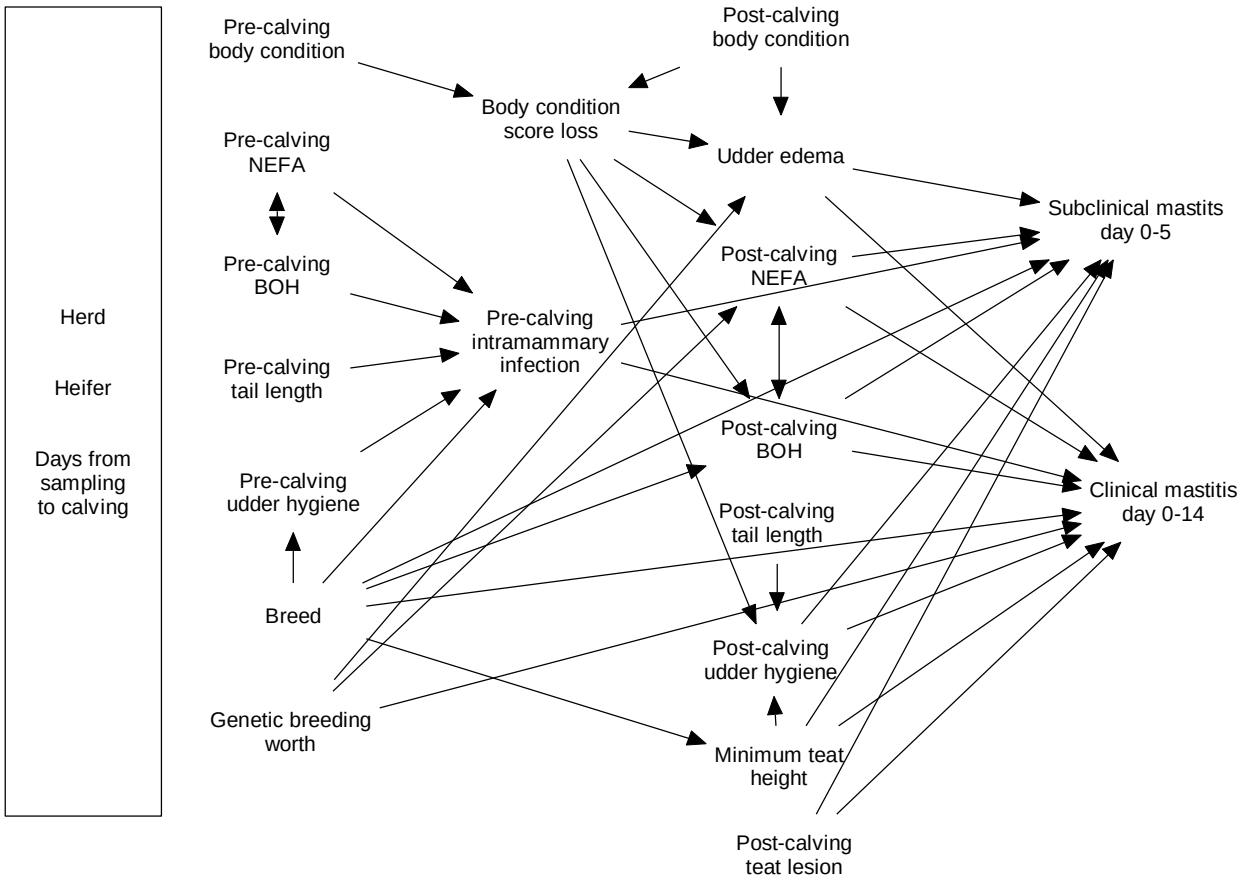
Within 5 days following calving, farmers were requested to present each enrolled heifer for collection of duplicate individual quarter milk samples and duplicate blood samples as previously, repeat recording of udder hygiene and body condition score, and recording of minimum height of the front quarters above the floor of the milking parlour, presence or absence of udder oedema Duplicate milk samples were also collected from quarters with the first case of CM diagnosed by the farmer within 14 days of calving. At approximately 3 months after the completion of the calving period, all enrolled heifers still in the milking herd were assessed by trained technicians for loss of function of any quarter and teats were palpated for evidence of thelitis.

Intramammary infection was defined as the presence of a recognized pathogen by bacteriologic culture of a milk sample, with or without signs of CM (quarters without clinical signs of mastitis were defined as SCM). Clinical mastitis was defined as presence of abnormal quarter secretion

including clots or serum-like secretion; or presence of heat or swelling or hardness of a quarter; as diagnosed by the farmer.

Many diseases of animals are multi-factorial. Path analysis enables researchers to postulate an ordering of causation between variables based on biological plausibility and temporal relationships, and estimate associations between them (Etherington et al., 1985). Path analysis methods have been applied to various veterinary epidemiologic studies including those on risk factors for postpartum disorders of dairy cows (Erb et al., 1985; Correa et al., 1993; Heuer et al., 2001). Path analysis methods are particularly appropriate for this study because of the temporal ordering of sampling and measurements, and the postulated effects of variables measured at the first visit on subsequent IMI and CM status. Path analysis was undertaken with methods described by Curtis et al. (1988). Study outcomes were modelled at the quarter level because bacteriological results were available on this basis, and variables at the heifer level were also included in a multi-level model. A hypothesized (null) path model (Figure 1) was formulated on the basis of findings from other studies in scientific literature, biological reasoning, or where there was an interest to test a specific hypothesis.

Figure 1. Null path model of hypothesized causal pathways between measured risk factors and subclinical and clinical mastitis in 708 pasture-grazed dairy heifers.



Data from this study were of a hierarchical nature (quarters nested within heifers, in turn nested within herds), and observations within the lower two levels of measurement could not be considered independent. To account for the correlation of IMI and CM of quarters within heifer and heifer within farm, each model included random effects for both, heifer and heifer nested in farm. The multilevel statistical model may be represented mathematically as:

$$(g)Y_{ijk} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_n X_n + \beta_{heifer\ (j)} + \beta_{herd\ (k)} + \varepsilon_{ijk}$$

where (g) refers to the logit link function, Y_{ijk} is the probability of the outcome variable on the logit scale, β's are the model coefficients, X's are the variables included in the models (days from calving to sampling when post-calving 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: $\beta_{heifer\ (j)} \sim N(0, \sigma^2_{heifer})$, $\beta_{herd\ (k)} \sim N(0, \sigma^2_{herd})$, $\varepsilon_{ijk} \sim N(0, \sigma^2)$.

Associations between variables were presented as incidence risk ratios as they explain the multiplicative risk of an outcome for a given level of exposure compared to a reference level. Incidence risk ratio measures and confidence intervals are 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). Non-significant paths were removed from the null model to give paths and coefficients for a final model (Figure 2) which shows only variables and pathways where significant direct effects were found or where confounding was considered important. The path coefficients represent the magnitude of the direct effect measured in units of incidence risk ratio of a hypothesized causal factor on an outcome factor, when all other predictive factors in the model are held constant.

Population attributable fraction (PAF) is defined by Dohoo et al. (2003, pg. 128) as the proportion of disease in the whole population that is attributable to the exposure, and would be avoided if the exposure were removed, assuming a causal relationship between exposure and disease.

$$PAF = \frac{p(E+)(RR-1)}{p(E+)(RR-1) + 1}$$

Where p(E +) is the prevalence of the exposure in the population, and RR is the relative risk (or incidence risk ratio). It is a useful measure to prioritize population health interventions, and is of most use when the factor of interest is clearly causally related to the outcome, and where the exposure is amenable to intervention. Point estimates and confidence intervals for PAF were estimated using the method of Greenland (Greenland, 2001) which uses adjusted risk ratios calculated from multivariable models and calculates confidence intervals using Wald estimates.

Statistical significance was declared for tests with P values $\leq$ 0.05, and confidence intervals for estimates are at the 95% level. Data was recorded in a Microsoft Access database, and statistical analysis was conducted using R version 2.2.0 (R Development Core Team, 2005).

Results

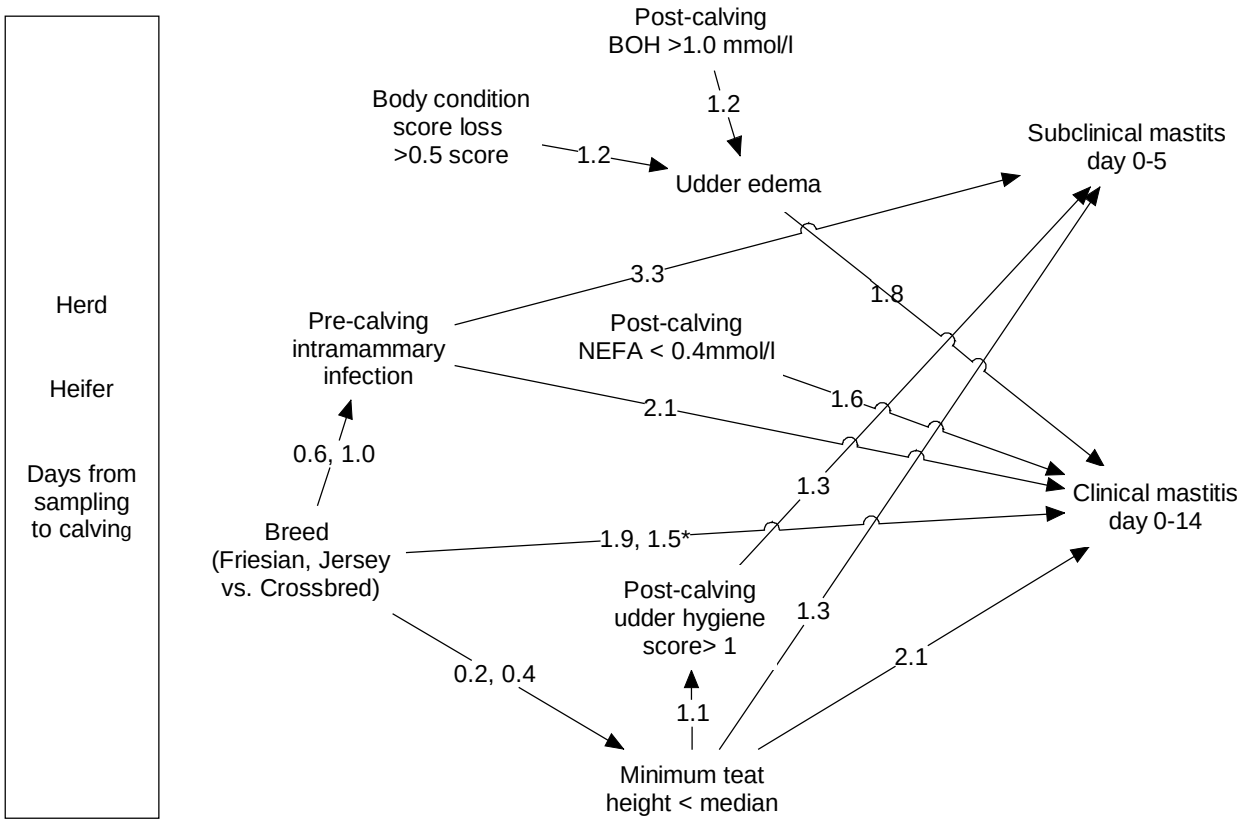
Pre-calving prevalence of quarters with IMI was 18.5%. Coagulase negative staphylococci were the most prevalent pathogen isolated (84.9%), and in decreasing proportion were *S. uberis* (15.5%), *S. aureus* (2.4%) and other gram negative bacteria (2.8%). Post-calving prevalence of IMI with any pathogen was 21.5%. Coagulase negative staphylococci were again the most common pathogen (54.7% of all pathogens), and in decreasing proportion were *S. uberis* (46.9%), *E.coli* (3.0%) and *S. aureus* (2.8%). Clinical mastitis was diagnosed in 7.0% of quarters and 23.4% of heifers within 14 days following calving. Pathogens isolated from quarters diagnosed with CM were mainly *S. uberis* (79.1% of all pathogens isolated), with lesser percentages of CNS (13.3%), *E.coli* (5.7%), *S. dysgalactiae* (3.8%) and *S. aureus* (3.2%).

Udder hygiene score >1 (mild to major contamination) increased significantly from pre- to post-calving (difference=17.9%, CI of difference=13.0% to 22.8%). Udder oedema was commonly diagnosed in heifers post-calving (prevalence=61%), and its prevalence significantly declined with

days post-calving. Median body condition score pre-calving of heifers was score 6, and the range was + 2 and – 1.5 units. Post-calving median condition score declined to 5, with a range of +/- 1.5 units. Serum BOH concentrations pre-calving had a median value of 0.7 mmol/l, but 21% of heifers had levels >1.0 mmol/l. Serum NEFA concentrations pre-calving had a median and upper quartile of 0.39 mmol/l and 0.56 mmol/l, respectively. Post-calving, 18% of heifers had serum BOH concentrations > 1.0 mmol/l, and serum NEFA concentrations had median and upper quartile values of 0.64 and 0.94, respectively.

Direct associations estimated as relative risk from regression analysis of variables in the final model are shown in the final path model (Figure 2). The associations of pre-calving IMI with SCM and CM were the strongest of all measured variables (RR=3.3 and 2.1, respectively). Estimates of energy status as a proxy for nutritional management were also significant in final models- post-calving NEFA < 0.4mmol/l was associated with an increase in the risk of CM of 60%, and post-calving BOH > 1.0 mmol/l increased the risk of EDEMA by 20% but was not directly associated with CM. Risk of oedema was also increased 20% by heifers who lost more than 0.5 body condition score (BCS) units, but BCS loss was not directly associated with CM. Post-calving hygiene scores >1 increased the risk of quarters being diagnosed with SCM by 30%, and heifers with minimum teat height less than the median had 30% and 110% increased risk of being diagnosed with SCM and CM, respectively.

Figure 2. Final path model for significant risk factors for subclinical and clinical mastitis in 708 pasture-grazed dairy heifers



* Coefficient for Jersey vs. Other breed on pre-calving intramammary infection not significant

Population attributable fractions for the outcomes SCM and CM are shown in Table 1. Presence of IMI in a quarter pre-calving had the largest PAF and smallest confidence interval of variables for SCM0_5. Three risk factors for CM0_14 had similar PAFs of approximately 30% (udder oedema, Friesian breed and minimum teat height less than the median), but that for udder oedema had the smallest confidence interval.

Table 1. Estimates of population attributable fractions for risk factors for subclinical and clinical mastitis in 708 pasture-grazed dairy heifers

		Population Attributable Fraction		
Outcome	Predictor	Estimate	LCL	UCL
SCM	PREBACTALL	0.30	0.26	0.34
	MINHGTLO	0.15	0.03	0.25
	HYGIENE_POSTPOOR	0.17	0.06	0.27
CM	PREBACTALL	0.17	0.10	0.24
	NEFAPOSTLO	0.27	0.19	0.35
	EDEMAPOST	0.33	0.15	0.47
	FRIESIAN	0.28	0.07	0.44
	MINHGTLO	0.37	0.16	0.53

Discussion

Udder oedema in heifers was associated with an 80% increase in the risk of CM cases- similar to that found by Waage et al. (2001) (teat & udder oedema, odds ratios with CM = 2.2 & 1.65, respectively) and Slettbakk et al (1995) (odds ratio = 1.35). A possible explanation for the association between oedema and CM is that the condition causes difficulty in milk removal during the milking process (Waage et al., 2001) and thus loss of the flushing effect, allowing pathogen numbers to increase above a threshold which overwhelm udder defence mechanisms. Elevated serum levels of BOH post-calving indicate lack of adaptation to high levels of body fat mobilization in response to inadequate dietary energy intake (Herdt, 2000), and may therefore only be diagnosed some period of time after onset of negative energy balance. The finding that elevated BOH and relatively high body condition score loss were directly associated with increased risk of udder oedema cannot be explained from data collected in this study. These associations would not have been identified if ordinary logistic regression techniques on CM as the outcome had been used, and demonstrate the value of path analysis techniques for exploring associations between antecedent variables. These associations have not been found by other workers (Al-Ani and Vestweber, 1986), so should only be viewed as tentative.

Udder hygiene has previously been found to be associated with the probability of environmental pathogen IMI (Schreiner and Ruegg, 2003). These authors found increased risk of IMI in cows with scores >2 compared with those • 2, whereas data from this study found a relationship post-calving existed with scores • 2 compared to score 1. Reasons for this difference might include the relatively small proportion of udders post-calving with scores • 2 (25%), and misclassification in measuring this variable because it was undertaken at the same time as milk sampling and may not have closely reflected udder contamination at the time of prior infection. A significant increase in udder contamination between pre and post-calving suggests grazing management or heifer behaviour differs between these periods, and because poor udder hygiene was associated with increased risk of IMI post-calving, management strategies to reduce this should be investigated. The finding of a small but positive association between udders of low minimum teat height and increased risk of poor udder hygiene might be explained by the reduced distance between the udder and the source of contamination (the ground) increasing the probability of contact by mud or water splash.

This study found increased risk of CM in heifers of Friesian breed (RR= 2.2) compared to Jerseys. A Scandinavian study (Myllys and Rautala, 1995) also found the risk of CM (+/- 7 days of calving) was higher in Friesian compared to Ayreshire heifers (5.6 vs. 3.9%, OR=1.6). Reasons for this were not found in this study, as Friesian breed was a significant direct risk factor for CM independent of the breed's increased risk for udder oedema and minimum teat height, and despite a lower prevalence of pre-calving IMI in Friesians than other Jerseys. However, the PAF of 0.28 for CM attributable to Friesians demonstrates that peripartum heifer management is particularly important for herds with a high proportion of this breed, and that the heritability of heifer mastitis among Friesian cattle should be evaluated to explore a possible genetic cause for the disease and sires sought with offspring that have greater resistance to IMI.

Pre-calving IMI or factors associated with it are important risk factors for both post partum SCM and CM. Myllys (1995) and Aarestrup and Jensen (1997) also found that quarters infected pre-calving had an increased risk of IMI post-calving than previously IMI-free quarters. In cows, the presence of a keratin "plug" in the teat canal is protective against new IMI over the non-lactating period (Smith et al., 1985; Dingwell et al., 2004). Prevalence of teat plugs in heifers before calving has not been reported in heifers. Presence of pre-calving IMI may be a proxy variable for absence of a teat plug, permitting colonization of the gland with the skin opportunist bacteria CNS. Open teat canals without teat plug defence mechanisms may be at higher risk of IMI in the last few weeks of gestation. This would be one explanation for the observed increase in the risk of infection in quarters with pathogens isolated pre-calving. An alternate explanation may be that IMI with minor pathogens themselves directly increase the susceptibility of quarters to subsequent infection, although evidence in support of this is in adult cows is conflicting (Hogan et al., 1988; Matthews et al., 1990). Pre-calving IMI with any pathogen had a stronger association with SCM (RR=3.3) than with CM (RR=2.1). This may possibly be due to the fact that only a small proportion of SCM infections actually become clinical, and that probably some cases of CM were not diagnosed (misclassified) by farmers, biasing the association towards the null.

Data from this study supports the view that factors influencing exposure to environmental organisms are important in determining post-calving IMI status. Firstly, bacteriological results showed the overwhelming importance of the environmental pathogen, *S. uberis*, in the establishment of new post-calving IMI and as a causative agent for CM. Therefore, risk factors that affect exposure to this pathogen are likely to be important in its transmission. Path analysis with this data showed that both low minimum height of teats above the ground and poor udder hygiene were positively associated with SCM and CM. Poor udder hygiene has been shown in mixed age cows to be associated with IMI due to environmental pathogens (Schreiner and Ruegg, 2003), hence high udder hygiene scores in heifers in this study were likely to also reflect an increased challenge from environmental pathogens to teat defences in this age group. It is also possible that heifers with lower minimum teat height were likely to have increased exposure to environmental pathogens because they were lower to the ground and more likely to be splashed with mud or faeces containing them (Lopez-Benavides et al., 2005). Slettbak et al. (1990) also found increasing teat end to floor distance was significantly protective of CM.

Heifer-level factors may also be associated with susceptibility to CM. It has been reported that increasing NEB as reflected in increasing serum NEFA concentrations depresses immune function (Adewuyi et al., 2005), but in this study the association was in the opposite direction. Although data to support the hypothesis was not collected in this study, a possible explanation for this might be that heifers in less negative or in positive energy balance (low NEFA post-calving) produced more milk immediately pre-calving, which had to be stored in the udder until the first milking, increasing intramammary udder and teat pressure and increasing the risk of loss of milk leakage. Therefore post-calving NEFA < 0.4 mmol/l may be a proxy variable for milk leakage pre-calving. Milk

leakage at calving was found to increase the odds (OR=1.36) of heifer CM on the day of or prior to calving (Waage et al., 1998), or between 1 and 14 days postpartum (OR=1.5) (Waage et al., 2001). Klaas et al. (2005) proposed that milk leakage provides a column of fluid through an open teat canal into the teat cistern and vehicle for infection of the mammary gland. In practical terms, data from this study suggests that the risk of CM may be reduced by permitting moderately reduced feed intakes of heifers immediately pre-calving through reducing the risk of oedema and possibly reducing the risk of milk leakage. Dietary intakes above maintenance should be avoided. Data from this study (not shown) suggests that the loss of <= 0.5 BCS units is associated with less udder oedema, but 'protective' levels of NEFA, and was not associated with decreased milk production in first or subsequent milk tests. However, nutritional management interventions need to be first tested in field trials before recommendations can be made.

Clinical mastitis is a primary concern of producers, so effective measures directed against oedema are most likely to reduce CM incidence. Pre-calving IMI (and/or factors associated with it) has a high PAF because of its high prevalence and relatively high incidence risk ratio for both CM and SCM, and therefore possibly has a higher priority in preventive strategies.

Path analysis techniques were valuable for proposing possible causal pathways for mastitis in dairy heifers, and permitted differentiation of factors associated with both exposure to pathogens and susceptibility to infection.

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