Epidemiology of Mastitis in Peripartum Dairy Heifers

A dissertation presented in partial fulfilment
of the requirements for the degree of

Masters of Veterinary Studies
in Epidemiology

at

Massey University
Palmerston North, New Zealand

by

Christopher William Raymond Compton

2006

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 determinants 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 weeks prior to the planned start of the calving period and within 5 days following calving, in addition to 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, *Streptococcus uberis* (*S. uberis*) prevalence increased four-fold to 10.0% of quarters. Prevalence of all pathogens decreased rapidly following calving. Clinical mastitis cases were predominantly associated with *S. uberis*. The hazard of diagnosis was higher in heifers than other parity groups combined and highest in the first 5 days of lactation. Intramammary infection was associated with an increased risk of removal from the herd and high somatic cell count (> 200 000 cells/ml) at subsequent herd tests, but neither CM nor IMI were associated with reduced milk yield or milk solids production. 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, 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 environmental exposure and host factors recommended.

Acknowledgements

Epidemiological studies in veterinary medicine collecting original field data require the involvement of many people to reach a conclusion, and this study is no exception. Throughout this study, I have been assisted, encouraged and challenged by many people. Dr. Scott McDougall, my mentor and colleague has been instrumental in not only starting me on this new career path, but also helping me complete this particular study. He has advised me on areas of study design and analysis, aided in logistic planning, and critically reviewed my work and writing. I also wish to acknowledge the encouragement and direction of my academic supervisor, Dr Cord Heuer. Further thanks go also to my colleague and fellow-student Dr Katrina Parker for her help in reviewing my written work.

I am also grateful to the 30 farmers and their staff who provided heifers and helped with sampling and data collection for the field study. My thanks also go to Animal Health Centre staff Fiona Anniss, Kathryn Berry, Rhonda Cooper, Elizabeth Blythe, Mike Kingstone, Shelley Roberts, Judith Forno and Helena Habgood, who carried out the on-farm data and sample collection. I also acknowledge funding from Dairy Insight (Project 20017) which enabled this study to be undertaken.

Approval for this study was sought and gained from the Ruakura Animal Ethics Committee (Approval No. 13548)

A special debt of gratitude is owed to my wife, Jane, and children Daniel, Jeremy and Rhys, who at times over the period of work on this dissertation have only had a part-time husband and father. They have encouraged me in my work, and given me the time needed to complete this project- time that they themselves were due. Thank you.

Table of Contents

List of Abbreviations

BTMSCC = bulk tank milk somatic cell count

CNS = Coagulase negative staphylococcus species

CM = clinical mastitis

IMI = intramammary infection

ISCC = individual somatic cell count

SCM = subclinical mastitis

List of Tables

List of Figures

Introduction

Mastitis or inflammation of the mammary gland in dairy cows is recognised internationally as a major problem facing dairy producers. Because of its importance, it has been, and continues to be the focus of substantial research effort. Much knowledge has been gained about the roles of milking and herd management, milking machine processes, and antibiotic therapies for the prevention and treatment of mastitis. However, more recently, mastitis in first-calving dairy heifers has been recognised as a separate problem, and international research efforts have been directed at describing the pattern and determinants of mastitis in this parity group.

This dissertation is comprised of 3 main parts. Chapter 1 consists of a review of scientific literature on mastitis in dairy heifers. This will provide a background of current knowledge, and shows that the epidemiology of mastitis in dairy heifers is distinct from that of other parity groups. It will explain why currently available mastitis preventive programmes are ineffective in heifers, and point towards research reported in this dissertation and that required in the future. The second and principal part consists of Chapter 2 and Chapter 3 which describe original research into mastitis in dairy heifers in New Zealand. Chapter 2 describes the pattern and consequences of mastitis both prior to and immediately following calving. Chapter 3 investigates risk factors that determine its occurrence, the likely contribution they make to the population incidence of disease, and suggests preventive strategies. The final part consists of the conclusion in Chapter 4, which summarises the principal findings from the earlier sections. It also gives the author's views of needs for future research and prospects for progress in the control of mastitis in dairy heifers.

Chapter 1- Literature Review of Epidemiology of Mastitis in Dairy Heifers

Introduction

Mastitis is a common infectious disease of dairy cattle. International surveys of dairy herds show the incidence of clinical mastitis to be among the highest of reported diseases (Bigras-Poulin, et al., 1990, Dohoo, et al., 1983). Population-based surveys of clinical disease in New Zealand dairy herds are few, but that which is available agrees with overseas literature on the high relative incidence of mastitis. Xu and Burton (2003) reported the overall clinical mastitis (CM) incidence of 12% per season (compared to lameness, 5.2%; calving difficulty, 3.3%; and metabolic disease, 1.8%).McDougall (1999) reported data from 38 commercial herds in early lactation with an average cumulative incidence of clinical mastitis (CM) of 9.9%.

In addition to being a common infectious disease in dairy herds, mastitis imposes significant costs on dairy producers (Seegers, et al., 2003, Wells, et al., 1998). Financial losses occur due to costs of treatment and prevention, loss of cows due to death and culling, loss of milk for supply during and after treatment with antibiotics, and potentially loss of milk production also. A review of the economics of mastitis and it's control by Schepers and Dijkuizen (1991) highlighted the variability in published estimates of the costs of CM depending on assumptions and methodology, but they are in the range of U.S $100-300 dollars per cow per lactation averaged over the herd. In contrast, a study of Ohio dairy producers (Miller, et al., 1993) reported total costs of prevention and losses from cases of CM to average U.S $52.41 per cow-year, and overall costs per CM case of U.S $107.11. A recent review of the costs of common diseases of dairy cows to the U.K industry (Bennett, et al., 1999) reported costs of mastitis to be approximately three times that of the next most costly disease (lameness).

Mastitis is defined as inflammation of the parenchyma of the mammary gland, regardless of the cause (Radostits, et al., 1984, pg. 563). There are physical, chemical and usually bacteriological changes in the milk and pathological changes in the tissue. Changes in the milk include the presence of clots, discolouration, and large increases in

the number of leucocytes. Changes in the udder tissue that may be detected include heat, swelling, pain and hardness. Where physical changes are detectable, mastitis is termed "clinical". Where physical changes are not detectable, but there are greatly increased numbers or leucocytes and/or pathogenic bacteria isolated from culture of milk, cases are termed "sub-clinical mastitis" (SCM). The causative agents of mastitis are predominantly bacterial, although fungal, yeast, algal and mycoplasma infections do occur. Infection of the udder (intramammary infection or IMI) occurs via the teat canal (except in the case of tuberculosis where the spread is haematogenous). This can originate from either another infected gland or teat skin via the milk liners or milkers' hands (contagious mastitis), or from the cows' environment such as bedding or faecal/mud material (environmental mastitis).

It is important that the characteristics of diagnostic methods used in the definition of a disease are known when a scientific study is undertaken (Dohoo, et al., 2003, pg.86). However, the test specifications for diagnosis of mastitis are not uniformly defined or implemented by workers in this field, and therefore results must be interpreted in the light of that variability. Because definition of clinical signs is subjective, and there is variability in the sensitivity with which these are detected, CM as an outcome variable is not uniformly defined either. Nevertheless, it is an important outcome as it defines the point at which treatment usually commences, and therefore at which direct costs are incurred. There are several ancillary tests used to aid in the diagnosis of SCM. The most important of these are the counting of epithelial cells and leucocytes (Dohoo, et al., 1981) recorded as individual somatic cell count (ISCC), indirect tests of udder inflammation such as electrical conductivity and the California or Rapid Mastitis Tests (CMT/RMT); and importantly, bacteriological culture of milk. Bacteriological examination by culture of milk samples is a standard diagnostic test for mastitis, and commonly accepted methodologies are described (Hogan, et al., 1999). Microbiological culture of milk samples however, also suffers from variable test sensitivity and specificity depending on several factors. These include procedures used for sample collection, handling and laboratory examination; pathogens involved; and stage of infection that the sample was taken at. Notwithstanding this, bacteriological culture provides evidence of the causative agent(s) of mastitis, and gives vital information for understanding the epidemiology of mastitis and formulating control programmes.

A large body of scientific literature exists on mastitis. Because of the high cost of mastitis to the dairy industry, vast resources have been used on research into its treatment and prevention. Seminal work on the control of infectious mastitis was published by workers at the National Institute of Research in Dairying, Reading, England (Neave, et al., 1966) and field studies of the results of control programmes published shortly thereafter (Kingwill, et al., 1970). This programme became known as the "Five point plan" and used an epidemiologic approach to reduce the prevalence of infection in the herd. It aimed to do this by both reducing the incidence rate of new infections through hygiene control, including segregation of infected animals, hygienic udder preparation, post-milking teat disinfection and milking machine maintenance; and the duration of infections by culling and effective therapy (especially by the introduction of non-lactating cow antibiotic therapy). Although this programme was successful (reduction of prevalence of infection in cows and quarters by 65% and 74% after 3 years, respectively) and gained widespread acceptance, it was acknowledged by its authors to have limitations (Kingwill, 1981). In particular, the incidence rates of new infections due to the "environmental" bacteria *Streptococcus uberis* (*S. uberis*) and coliform types were only slightly reduced. ("Environmental" mastitis was defined by Smith et al (1985a) as "those IMI caused by pathogens whose primary reservoir is the environment in which the cow lives and not infected mammary quarters.").

The majority of the research into mastitis in dairy cows has involved adult cattle, and only a small proportion has specifically involved dairy heifers. Although much of the findings from this research (especially pathology and immunology) will relate to heifers, this literature review will demonstrate that there are distinct differences in the epidemiology of mastitis in heifers as compared to multiparous cows, requiring a separate appraisal of mastitis in this younger age group. These differences may be attributed to several factors- including that pre-partum heifers have not been milked, undergone an udder involution or dry period, and have been reared separately from adult cows in different environments. Therefore heifers have different exposure and protective factors which influence the epidemiology of disease in this parity group.

This review will consider literature published from 1970 onwards, in the English language, found by searching electronic databases. Publications of original scientific research in peer-reviewed journals are emphasised, where the principal aims of the

workers were to describe the epidemiology, prevention, treatment and economic cost of mastitis in dairy heifers, especially that occurring in the pre- and early post-parturient period. More emphasis is given to recent publications because both the heifers studied and management systems under which they were farmed are likely to be more similar to current animals and practices than those from earlier studies. This makes their findings more applicable to modern dairy systems. Studies reporting findings from the pre- and early post-parturient periods of heifers are concentrated on because, as it will be shown, these are the periods of highest risk of new infection and also therefore give the greatest opportunities for mastitis control.

Descriptive epidemiology of heifer mastitis

Despite reports of CM in heifers in the periparturient period from early in the 1900's, it was initially believed that heifers were free of IMI prior to calving (Myllys and Rautala, 1995, Oliver and Sordillo, 1988, Shearer and Harmon, 1993). This may have been due to the belief that because contagious pathogens are transmitted primarily during the milking process, heifers at first calving would not have been exposed to at least these bacteria, and hence would be uninfected (Oliver and Sordillo, 1988).

Clinical mastitis and IMI in dairy heifers have been recognised as important problems with infection patterns different from other age groups for at least 35 years. Munch-Peterson (1970) described in a longitudinal study in 3 herds that 22.2% of quarters from 134 heifers were infected at the first milking, reducing to 9.4% by the 7th day. He noted also that infection persisted in 6.3% of quarters beyond the first week of lactation. Work by Barkema et al. (1998) emphasised 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. Zecconi et al. (2003) found heifers to have new infection rates with S. aureus in the first 30 days of lactation (2.2 cases/100 cow months) approximately twice that of other parity groups. A survey of bovine mastitis treatments in Nordic countries (Valde, et al., 2004) found higher cumulative risks for Parity 1 cows compared to Parities 2 and 3 in the first 2-3 weeks of lactation, but there was variability between countries. A summary of results from reviewed surveys and analytical studies giving incidence and prevalence data are

shown at the end of this section in Table 1 and Table 2. Where possible, data are shown as reported by authors, but some were recalculated for display. Studies used different sampling, microbiologic, and statistical methods, and hence caution must be taken in making comparisons between different studies.

Bacteriologic testing of milk samples from heifers taken at varying time relative to calving and managed under different systems showed different prevalence of bacterial species. A survey of IMI at calving in two Irish spring-calving herds (Meaney, 1981) wintered under confinement and pasture-grazing systems, found 42% and 22%, respectively of heifers infected at calving. Sixty percent and 72% of infections in confined and pasture-grazed heifers, respectively, were due to environmental Streptococcus spp. and *Escherichia coli* (*E. coli*), and 90% of IMI had clinical signs. In contrast, a Norwegian bacteriological survey from cases of CM in heifers prior to or within 14 days of calving (Waage, et al., 1999b), found a high prevalence of *S. aureus* (45.8%), with *S. dysgalactiae* and coagulase negative Staphylococci (CNS) the next most prevalent (19.9% and 12.8%, respectively). They found that the relative proportions of bacterial species were similar for both pre- and postpartum CM (except for *Arcanobacterium pyogenes*, which was more prevalent prepartum or on the day of calving due as an expression of "summer" mastitis in that country), and that bacteria species prevalence varied significantly by month of the year and region.

Several studies reported on relationships between pre- and postpartum IMI patterns to determine time and persistence of infection. A brief report from Cooper, Buddle, et al. (1977) on mammary gland secretion samples from N.Z. heifers 2-6 weeks prior to calving found 22%, 91% and 10% of quarters infected with haemolytic Staphylococci, non-haemolytic Staphylococci and Streptococci, respectively, and that some of these persisted throughout the following lactation. A longitudinal study in 32 primigravid heifers over the periparturient period (Oliver and Mitchell, 1983) found 33% of quarter samples with an IMI (11% of quarters with a major pathogen) pre-calving, 31% at calving, but a markedly reduced percentage of quarter IMIs (especially with CNS) in the first 14 days of lactation (12.7% total). They concluded that pathogens of environmental origin (non-agalactiae Streptococci and coliforms) were the predominant major intramammary pathogens of heifers at calving, they were present at least 2 weeks before calving, and that control and eradication of *S. aureus* and *S. agalactiae* from adult

milking cows did not eliminate mastitis problems in heifers. A similar pattern of infection with a larger sample of heifers from the same group of workers (Oliver and Sordillo, 1988) supported their earlier findings on major pathogens, and showed that CNS bacteria were the predominant isolate over this peripartum period (55.7%), followed by non-agalactiae Streptococci (20.4%) and coliforms (15.3%).

Already at this early stage after the recognition of heifer mastitis as a problem, essential knowledge of pathogens, prevalence and temporal relationships existed, but more knowledge was required on their ecology, and the pathology associated with IMI. A 1985 longitudinal study was undertaken into the prevalence of infection on teat skin, streak canal and mammary secretion in 10 Jersey heifers in Louisiana from the age of first breeding to calving (Boddie, et al., 1987, Nickerson, et al., 1995). They found 54.1%, 70.1% and 86.1% of samples from teat skin, streak canal and mammary secretion, respectively, infected with either Streptococcal or Staphylococcal species. Coagulase negative staphylococcal (CNS) species *S. xylosus* and *S. chromogenes* were the predominant skin isolates, and *S. chromogenes,* followed by *S. hyicus* and *S. aureus* were the most prevalent in both streak canals and mammary secretions. Evidence of inflammatory response to these mammary secretion isolates was shown by 2-3 fold elevation of somatic cell count of pre-calving secretions as compared to uninfected quarters. Histological examination showed inflammatory changes in teat canal tissues in the presence of teat duct colonisation, and in udder parenchyma of infected quarters. These workers contended that teat skin, canals and mammary secretions were infected with mammary pathogens from an early age, and that these infections could persist for up to one year.

Studies on the ecology of heifer intramammary pathogens, particularly the more prevalent CNS species, showed that species prevalence varied over time, by site sampled, management conditions and location. White et al. (1989) conducted a survey in Kentucky of Staphylococcal infection of nulliparous dairy heifers from several body sites (including streak canals) and managed under differing conditions. *Staphylococcus xylosus, S. chromogenes* and *S. warneri* were isolated most frequently and were more likely to be cultured from older and pasture-grazed heifers. The percentage of Staphylococcal isolates from the teat skin and streak canal was similar, with *S. chromogenes* and *S. warneri* the predominant streak canal species. Another study on

the prevalence of IMI and teat canal colonisation in unbred and primigravid Jersey dairy heifers from four Louisiana herds (Trinidad, et al., 1990a) found IMI in 74.6% of quarters and 96.9% of heifers, with CM prevalence of 15.1% of quarters and 29% of heifers. *Staphpylococcal* species predominated in the samples- *S. chromogenes, S. aureus, S. hyicus* (35%, 22% and 17%, respectively). Teat canal colonisations were found in 70.7% of quarters and 93.1% of heifers. An important finding from this study was the high prevalence of the major udder pathogen, *S. aureus*, in both teat canal and udder secretions. The authors proposed preventive measures for heifer mastitis including more effective teat spraying post-milking of adults to remove pathogens on the skin and better control of flies (later controlled by insecticide-impregnated ear-tags) to reduce insect-borne transmission of these bacteria, and the use of intramammary antibiotics in unbred and primigravid heifers.

Findings of high prevalence of IMI with major pathogens prepartum in Louisiana heifers stimulated surveys of IMI prevalence in other regions of the U.S.A. (Fox, et al., 1995, Matthews, et al., 1992, Pankey, et al., 1991). A New Zealand study in pasture-grazed heifers (Pankey, et al., 1996) found 35% of heifers with an IMI within 5 days of calving, and that CNS were the most prevalent isolate (21.8% of heifers), followed by environmental Streptococci (almost exclusively *S. uberis* 12.2%), *S. aureus* (0.9%) and coliform species (0.7%). A Danish longitudinal IMI prevalence study (Aarestrup and Jensen, 1997) found that the CNS organism *S. chromogenes* was the only bacteria isolated 4 weeks pre-partum, was the most prevalent isolate overall, and had a peak prevalence of 15% of quarters 1 week prior to calving. Of interest is their finding that a high proportion of prepartum *S. dysgalactiae* infections were found to persist after calving (confirmed using bacterial ribotyping tests). Although the pattern and relative importance of pathogens differed between regions, overall, these studies together found that CNS prevalence was highest prepartum and that environmental pathogen prevalence peaked at calving.

Table 1. Cumulative incidence (%) of clinical mastitis in quarters and heifers (bold type) in periparturient heifers.

	Period relative to calving			
Bacteriological results	All weeks pre-calving	Calving	0-1 week post-calving	1-2 weeks post-calving
*S. aureus**	3.3[4]			
Streptococcus spp.	1.3[4]	**5.5**[3]		
CNS species	6.6[4]	**0.9**[3]		
All species	15.1[4] **29.0**[4]	**8.1**[3],13.0[2], **31.2**[2]	**10.1**[1]	**1.5**[1]

* *Staphylococcus aureus*

Key of authors:

1 (Barnouin and Chassagne, 2001), 2 (Meaney, 1981), 3 (Pankey, et al., 1996), 4 (Trinidad, et al., 1990a). For longitudinal studies, bacteriological results should be read across row by same superscript for comparisons over time.

Table 2. Prevalence of intramammary infection by bacteriological species in quarters and heifers (bold type) in periparturient heifers.

	Period relative to calving			
Bacteriological results	2-0 weeks pre-calving	All stages pre-calving	Calving	1-2 weeks post-calving
S. aureus [a]	0.5[8], 0.7[5], 1.2[4]	2.9[7],13[1], 14.9[6], **37.1[6]**	0.7[5], 0.7[9], 0.8[4], 1.3[3], 2.8[7], 6.7[8], 7.6[2], **0.9[10]**	0.4[4], 0.5[5], 2.7[3], 3.5[2], 6.7[8]
CNS [b]	15.7[5], 22.2[4], 26.9[8]	27.1[7], 53.2[6], 71.7[1]	11.4[9], 14.4[5], 17.8[8], 18.8[4], 21.8[7], 23.7[3], 27.9[2], **21.8[10]**	6.4[5], 7.5[4], 7.6[3], 18.1[2], 12.1[8]
S. agalactiae [c]			1.9[3]	5.5[3]
S. dysgalactiae [d]	1.8[8]	0.3[6]	1.0[3], 4.9[8]	1.4[8]
S. uberis [e]	2.0[8]	1.4[1]	2.1[8],4.2[3]	1.1[8],3.4[3]
Streptococcus spp. (non-*agalactiae*)	3.4[5], 4.8[4],	3.5[6]	2.6[9], 7.4[5], 7.8[4], **12.2[10]**	2.4[4], 3.7[5]
Coliforms	3.0[5], 4.8[4], 1.1[8]		0.7[8], 2.2[9], 4.3[5], 4.7[4], **0.7[10]**	0.5[8], 2.4[4], 2.6[5]
Environmental		1.5[7]	7.7[7]	
Coryne. bovis [f]	0.4[5]		0.8[4], 1.0[5]	1.2[5]
All species	23.9[5], 32.0[8], 33.0[4]	31.0[4], 34.4[7], 74.6[6], **96.9[6]**	18.6[9], 28.1[5], 36.0[7], 36.9[8], **31.7[10],45.5[9]**	12.7[4], 14.5[5], 24.7[8]

Key of authors:

1 (Boddie, et al., 1987), 2 (Matthews, et al., 1992), 3 (Munch-Petersen, 1970), 4 (Oliver and Mitchell, 1983), 5 (Oliver and Sordillo, 1988), 6 (Trinidad, et al., 1990a), 7 (Fox, et al., 1995), 8 (Aarestrup and Jensen, 1997), 9 (Pankey, et al., 1991), 10 (Pankey, et al., 1996).

For longitudinal studies, bacteriological results should be read across row by same superscript for comparisons over time.

Abbreviations of bacterial species:

[a]*Staphylococcus aureus,* [b]Coagulase negative staphylococci, [c]*Streptococcus agalactiae,* [d]*Streptococcus dysgalactiae,* [e] *Streptococcus uberis,* [f]*Corynebacterium bovis*

Risk factors for heifer mastitis

Following on from, and sometimes concurrently with surveys, researchers investigated risk factors associated with heifer peripartum mastitis. These studies formed a basis for recommending preventive programmes, and provided knowledge to direct new research.

The investigation of herd-level risk factors for a disease requires analysis of data from large numbers of herds to provide statistical power to show significant differences between studied factors. National databases recording dairy cow disease and production provide such a resource and hence, several Scandinavian authors have analysed such data to estimate both herd and individual heifer risk factors for heifer peripartum mastitis. Myllys and Rautala (1995) found a significant association between the cumulative incidence of CM in heifers within 7 days prior to or after calving and herd milk production (5.8 vs. 3.5% for high (> 7000 kg/yr) and low (< 6000 kg/yr) mean production herds, respectively). There were also higher odds in herds with low bulk tank milk somatic cell count (BTMSCC), high incidence of CM in other parity groups, and good nutritional management. A Norwegian study (Waage, et al., 1998) also found significant associations between heifer CM on or before the day of calving and herd incidence rate of mastitis (OR = 1.43 if > 20 cases/100 cow years), BTMSCC (OR = 0.84 for each increase of 10^5 cells/ml), herd size (OR = 0.49 for herds > 30 cow-years), herd average per cow milk yield (OR = 1.1 for each increase in average yield of 500 kg of fat and protein corrected milk, or FPCM), region within country, and feeding and grazing management. Conversely, de Vliegher et al. (2004) found that using high ISCC 5-14 days post-calving as a measure of postpartum IMI, heifers with low ISCC were more common in high-producing herds with low BTMSCC.

Positive associations between milk production and CM have been found from several studies. Individual cows and heifers diagnosed with CM from one observational study (Grohn, et al., 2004) tended to have higher milk production prior to diagnosis than non-CM cows of the same parity within a herd . A review of mammary gland immunity and mastitis susceptibility by Sordillo and Streicher (2002) stated that a negative correlation exists between milk production capacity and resistance to mastitis. They also noted that changing management systems and increasing cow densities may increase exposure to environmental mammary pathogens. A possible explanation for the apparent association

between high incidence of heifer CM and high incidence of CM in cows and low herd BTMSCC may be that some farm managers aggressively detect and treat CM and SCM in all age groups of cows (causing high incidence of CM), and withdraw milk from such cows from saleable supply until subclinical signs e.g. RMT scores have normalised (causing low BTMSCC). This would be an example of reverse causation (Dohoo, et al., 2003, pg.145), and is difficult to control in cross-sectional studies when the exposure and outcome are measured at the same time.

Individual heifer risk factors have been analysed using both national database records and data gathered from purposively-designed cohort and case-control studies. A Scandinavian study (Myllys and Rautala, 1995) using national database records, found the cumulative incidence (CI) of CM (+/- 7 days of calving) differed significantly between Friesian and Ayreshire heifers (5.6 vs. 3.9%, OR = 1.6). Age of animals has also found to be associated with heifer CM. A Swedish study (Oltenacu and Ekesbo, 1994) of CM in heifers found those calving at approximately 2 ½ years of age had lower odds of CM within 50 days of calving than older or younger heifers, and a Belgium study (de Vliegher, et al., 2004) reported lower ISCC in heifers with calving age > 27 months. In contrast, Waage et al. (1998) and Erb et al. (1985), found an increase in odds of CM on the day of or prior to calving or throughout lactation, respectively, with increasing age at calving (OR = 1.29 for each increase in month, and not reported, respectively). Month of calving has also found to be associated with heifer peripartum CM (Oltenacu and Ekesbo, 1994, Waage, et al., 1998), but specific reasons for the temporal pattern were not given by the authors.

Other diseases associated with parturition have also been found to increase the risk of heifer peripartum CM. Dystocia was found to increase the odds of CM in heifers 2.2 and 1.9 times (Barnouin and Chassagne, 2001, Oltenacu and Ekesbo, 1994), respectively. Oltenacu and Ekesbo (1994) also found that heifers with retained placenta had 1.7 times increased odds of having CM within 50 days of calving. Causal mechanisms involved with these associations are unproven, but might be related to increase in exposure to environmental pathogens due to prolonged recumbency (with dystocia), or contamination of teats (from the retained placenta). Changes in immune function such as might occur with hyperketonaemia are proposed to increase

susceptibility to IMI (Suriyasathaporn, et al., 2000) but have not been directly proven in peripartum heifers.

Several workers have specifically examined relationships between udder and teat characteristics and heifer peripartum CM. Increased milk-out speed was associated (OR = 1.1 per kg milk per 2 minutes) with mean ISCC (Slettbakk, et al., 1990), but the association was marginally significant (OR = 1.4) for CM throughout lactation (Slettbakk, 1995). Milk leakage at calving was found to increase the odds (OR = 1.4) 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). Other important risk factors for CM in heifers between 1 and 14 days postpartum found by Waage et al. (2001) included teat & udder oedema (OR = 2.2 & 1.65) and blood in milk (OR = 3.4). Slettbakk et al. (1995) found a similar association between udder oedema (OR = 1.35) and CM (throughout lactation), as well as that increasing teat end to floor distance was significantly protective of CM (52-55cm OR = 0.76, > 55cm OR = 0.59, vs. < 52cm). These factors were hypothesised to increase exposure of the glands to IMI by increased diameter of the teat canal (milking speed), increased length of time the teat canal is open prior to calving (milk leakage), reduced efficiency of milk and pathogen removal (udder and teat oedema), and increased susceptibility to damage and contamination (low teat height).

Another area of debate amongst mastitis researchers is the association between pre-calving gland IMI bacterial status and post-calving risk of IMI. In a longitudinal study of 50 heifers in Finland, Myllys (1995), found that quarters infected pre-calving were more susceptible to IMI post-calving than were previously IMI-free quarters. A longitudinal study involving 180 heifers from 20 herds in Denmark (Aarestrup and Jensen, 1997), involving weekly sampling all quarters from 4 wks pre to 4 wks post calving, found significant associations between prepartum IMI with CNS, *S. uberis* and *S. dysgalactiae,* and IMI postpartum with any bacterial species (except for *S. aureus)*. These findings are in contrast to recent work by de Vliegher et al. (2003) who reported that teat apex colonisation of heifers with *S. chromogenes* reduced the odds of somatic cell counts greater than 200,000 (as an indicator of IMI) at days 3-5 of lactation, but detailed bacteriological results were not given and neither was day of sample collection postpartum controlled for in analysis.

The possible risk of IMI in pre-weaned calves from feeding mastitic milk to calves has been a concern to farmers and veterinarians. Barto et al. (1982) carried out a longitudinal experimental study to determine the risk of *S. aureus* IMI in postpartum heifers in a challenge study where calf milk was spiked with the organism and calves were reared in individual pens. There was no statistical difference in the number of heifers infected between treatment and control groups, although the study size was small (64 heifers sampled at calving). A review published near that time (Kesler, 1981) concluded that although data were limited, calves fed mastitic milk suffered no increase in udder disease.

Prevention of heifer mastitis

Using knowledge that heifer IMI prevalence may be moderate to high pre-partum, several randomised controlled field studies were carried out testing various intramammary antibiotic treatment regimens pre-partum for prevention of heifer periparturient mastitis. Trinidad et al. (1990b) reported a Louisiana study involving 73 heifers from three herds with almost 100% prevalence of IMI. A penicillin and dihydrostreptomycin non-lactating cow intramammary treatment markedly reduced post-calving quarter IMI prevalence (52% in previously infected control quarters to 7% in treated quarters), and reduced SCC at first milk production test. They found the greatest impact of treatment was when it was administered in the second trimester of pregnancy. A Louisiana study (Owens, et al., 1994) in 75 research & 40 commercial heifers used a cephapirin non-lactating cow therapy 10-14 weeks pre-partum and recorded greater than 90% cure rates of existing IMI. They also reported prepartum non-lactating cow therapy cure rates higher than lactating cow therapy at calving for *S. aureus* IMI (96% vs. 62.5%). A follow-up study by this same group (Owens, et al., 2001) with 233 heifers used 5 different non-lactating cow antibiotics in each trimester. Overall there was no difference between products and stage of pregnancy with respect to cure rate (80-100%), but fewer new infections persisted until calving after treatment in the last trimester. They therefore recommended treatment 60-45 days prepartum to give sufficient time for antibiotic residues to decline and to ensure the maximum number of infections is treated. Significantly improved milk production quality resulting

in economic benefits have been demonstrated following use of prepartum antibiotic therapy in heifers (Oliver, et al., 2003).

In addition to non-lactating cow intramammary antibiotics, studies have been conducted with treatments normally used for lactating cows, administered closer to calving. Oliver et al. (1992) used different antibiotics (lactating cow intramammary cloxacillin and cephapirin) administered seven days prior to calving and found treatment eliminated 83% and 97 % of all IMI respectively (compared to 29% self-cured). A later study (Oliver, et al., 2004) used penicillin-novobiocin and pirlimycin treatments 14 days before due calving date to significantly reduce IMI prevalence in treated heifers post-calving.

Usage of intramammary antibiotics designed for lactating cows or those at their last milking, instead in heifers prior to calving, does not follow manufacturer recommendations. Such treatment would be expected to increase the risk of heifers producing milk with antibiotic residues in early lactation. Consequently, this risk has been assessed by several workers, with varying results according to the products and the time of use before calving. Trinidad et al. (1990b) found only 3% of quarters treated with penicillin and dihydrostreptomycin 3 months prior to calving had antibiotic residues within 3 days of calving, and concluded that this risk was low. A further study (Oliver, et al., 1992), found 17% of composite milk samples tested positive on the day of calving (0% subsequent to that) for antibiotic residues in heifers treated with lactating cow cloxacillin approximately 7 days pre-partum, but that 85 %, 28% and 0% were positive at 0, 3 and 10 days post-partum, respectively, following cephapirin treatment. One further study (Owens, et al., 1994) reported no antibiotic residues detected at 5 days postpartum following cephapirin non-lactating cow therapy given 10-14 weeks earlier.

Only one report of an intervention study using a non-antibiotic peripartum mastitis preventive strategy for heifers was found. In a study of all parity groups, Edinger (2000) reported no significant protective effective against IMI from using an iodine-based barrier teat dip starting at an estimated 12 days prior to expected calving. However, the study was conducted in only one herd, major pathogens were mainly *S. aureus* rather than environmental pathogens, and the statistical power to show a significant reduction

in IMI incidence with only 149 heifers was low. However, a series of two unpublished field studies (K. Parker, personal communication) showed significant protective effect from using an internal teat sealant (bismuth subnitrate) used from 3 weeks prepartum against CM and IMI in pasture-grazed dairy heifers.

Long-term effects and economic cost of heifer mastitis

Knowledge of whether heifers diagnosed with CM or IMI are more likely to have recurrent infections, other problems, or are more likely to be culled, is important for economic analysis of treatment and control programmes. These costs are additional to direct treatment costs and should be quantified. Waage et al. (2000) reported on 1 month follow-up of treatment of CM cases in 1000 heifers in Norway. Heifers with CM prior to or within 14 days postpartum had 2.3 times the risk of being culled within 28 days of treatment than non-CM heifers (10.5% vs 4.5%), 25% of CM-heifers had one or more non-functional glands, 17% had a subclinical infection, and 14% were still clinical. They reported high proportions of non-functional quarters following *Arcanobacter pyogenes* or *S. aureus* infection, and that thelitis was common in heifers with chronic mastitis. An increase in the risk of culling following heifer CM was also shown by Erb et al. (1985) (5.2 times risk for first diagnosis at any time in lactation), Myllys & Rautala (1995) (8.6 vs. 4.6% in non CM heifers for first diagnosis with 7 days pre- or postpartum), Oltenacu and Ekesbo (1994) (2.8 and 2.4 fold increase in odds for heifers with CM prior to or within 50 days following calving, respectively), and De Vliegher et al. (2005a) (11% increase in the daily hazard of culling in heifers for each unit increase in early lactation log-transformed ISCC).

Several problems arise in estimating the economic cost of heifer mastitis. Firstly, published reports on the economic cost of mastitis in heifers are few in comparison to those on mastitis in multiparous cows, providing a small range of estimates. Further adding to uncertainty, the methodology used varies between studies, and this influences estimates of production losses, and needs to be considered when estimating the total economic cost of heifer mastitis. A central problem in this estimation are the methods used to deal with the confounding effect of milk production on risk of mastitis (Grohn, et al., 2004, Rajala-Schultz, et al., 1999). 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; when in fact the true loss is the difference between the (higher) unmeasured potential production and what was actually measured. In multiparous cows, 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. But this is not possible in heifers in their first lactation, and either predicted genetic ability for milk production traits as a proxy variable for production potential, or measured production prior to CM, may be used as a covariate.

Decreased milk production in heifers following IMI or CM has been occasionally reported, with estimates in the range of approximately 10% or less for an entire lactation. An experimental challenge study in a split twin herd of all age cows (Woolford, et al., 1983) produced an infected herd with an average *S. aureus* quarter infection rate of 1.6 quarters per cow. This was an efficient study design to estimate production losses due to IMI, because genetic ability for milk production was balanced between infected and uninfected groups (one of two twins allocated to each group). Infected heifers showed a 7.8% loss of milk production (213 litres) compared to uninfected heifers. The same authors went on to report an 8.4% loss of milk yield in the second lactation of previously infected but cured heifers (Woolford, et al., 1984). Another experimental challenge study (Owens, et al., 1991) reported heifers with one or more persistent *S. aureus* infections post-calving averaged 11% lower production than cured heifers. Rajala-Schultz et al. (1999) analysed database records and reported production losses of 4.7% in first lactation heifers with CM diagnosed before peak lactation. De Vliegher et al. (2005b) estimated production losses of 119 kg for heifers with ISCC of > 500,000 at day 10 of lactation compared to heifers with ISCC < 200,000 cells/ml.

Milk production losses of this magnitude due to IMI of heifers have not always been found however, in other field studies. Jones (1984) used elevation of ISCC (as an indicator of IMI) to estimate milk yield, and found losses increasing from 0.13 kg/day for counts above 200 000, and that losses in older age cows were approximately twice that of heifers for the same ISCC. Myllys & Rautala (1995) estimated seasonal milk

yield loss of 70 kg yield for heifers with CM within 7 days pre- or postpartum. In contrast, heifer CM at any stage in lactation was found to have no effect on mature equivalent milk yield (Barnouin and Chassagne, 2001, Erb, et al., 1985). Possible reasons for differing results between experimental and field studies might include increased severity and duration of *S. aureus* IMI in these experimental studies compared to less chronic and more readily-cured infections in field studies. Milk production may have also been more precisely measured in experimental studies permitting significant effects to be demonstrated. A field study in 2 herds using daily milk production recording data (Grohn, et al., 2004) found greatest milk production losses from CM cases where *S. aureus* and gram negative organisms, *Arcanobacterium pyogenes* or no pathogens were isolated. Streptococcus spp. infections caused significant production losses for only one week after diagnosis and treatment.

Conclusions

Clinical mastitis and IMI in periparturient heifers are common in dairy farm systems worldwide, and their incidence and prevalence vary widely. Intramammary infections are mostly subclinical prepartum, but more likely to be clinical in the early postpartum period. Bacterial pathogens involved in these infections weeks to months pre-calving are predominantly minor skin-associated bacterial species, but major environmental IMI pathogens become more prevalent in the week prior to and a few days immediately following calving. All except contagious pathogens decrease in prevalence as lactation progresses. Sources of infection for heifer peripartum mastitis are mainly environmental because of the limited or absence of exposure to adult cattle and the milking process. It is likely that many new IMI occurring immediately prepartum and are subsequently diagnosed as CM and IMI at or after calving.

Several risk factors for heifer peripartum mastitis and outcomes have been reported. However, patterns of heifer peripartum mastitis, production and culling vary with management and location. Most reported data has been gathered from heifers managed under mainly confinement systems, which may not be applicable to, or for which other factors may operate, compared to pasture-grazing systems as used in New Zealand. Therefore there is a need to conduct field studies under New Zealand farming systems

with its own unique characteristics to describe patterns of IMI and CM in peripartum heifers, and to identify and quantify their risk factors. Also, there are few reports of production losses resulting from heifer CM and IMI, especially in pasture-grazed dairy heifers, which means the total cost of heifer mastitis can only be imprecisely estimated for economic analysis of any suggested control programme. Provision of accurate estimates of any production losses due to heifer mastitis would provide a basis for economic analysis of recommendations for preventive measures.

Current widely-used mastitis control methods which were developed for contagious mastitis in cows in milk have not been found to be effective against heifer mastitis, in which the source of infection is environmental, and often where infection already exists before the first milking. Currently, the only successful preventive programmes published specifically for heifer mastitis, have been based on prepartum intramammary antibiotic therapy, but prophylactic use of antibiotics may not be politically acceptable, practicable, or economically justifiable under N.Z conditions. Hence there is a need to develop alternative preventive programmes based on non-antibiotic methods, and particularly those that can be applied at the heifer group level by management interventions.

Chapter 2- Descriptive Epidemiology of Mastitis in Pasture-Grazed Peripartum Dairy Heifers and its Effects on Productivity

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 (Barkema, et al., 1998, Barnouin and Chassagne, 2001, Pankey, et al., 1991). A high prevalence of IMI has also been reported in heifers before calving (Trinidad, et al., 1990a, White, et al., 1989), and a positive association has been found between pre- and postpartum infection (Aarestrup and Jensen, 1997, Matthews, et al., 1992, Oliver and Sordillo, 1988). 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).

The impact of peripartum mastitis in heifers on productivity and longevity has been examined by several authors. Estimates of milk yield losses in heifers diagnosed with CM are variable, with studies demonstrating no loss (Barnouin and Chassagne, 2001, Myllys, 1995), up to 6% loss (Hortet and Seegers, 1998), 5% loss (Oltenacu and Ekesbo, 1994), 8.4% loss (Woolford, et al., 1983), and as high as 7kg milk per day for several weeks following CM (Grohn, et al., 2004). Additionally, some heifers never regain their production potential (Grohn, et al., 2004). The risk of culling also increases 2- to 3-fold for heifers with CM early in lactation (Myllys and Rautala, 1995, Oltenacu and Ekesbo, 1994, Waage, et al., 2000).

First-calving heifers represent a valuable current and future resource. They make up the largest parity group in most herds, they have the highest genetic merit of any age group in the herd (Livestock Improvement Corporation, 2005) and, until a calf or milk is sold following their first calving, have not contributed to income for their owner. For these reasons, diseases which are frequent, and might adversely affect the animal's production and lifetime performance are of concern to dairy producers.

Little information 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 are 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.

Mastitis preventive programs currently advocated have been developed mainly to control infections due to contagious pathogens in cows once they are being milked or are in their non-lactating period (Kingwill, et al., 1970), and do not specifically address the problem of IMI in peripartum heifers. Measurement of the prevalence and incidence of an infectious disease, the organisms involved, and patterns of infection, is a necessary first step in disease investigation (Dohoo, et al., 2003, pg. 66), and ultimately formulation of control programs. Estimates of lost production and increase risk of premature removal from the dairy herd due to a disease are also necessary to formulate cost-benefit analyses of proposed preventive programs.

Hence, 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. Additional aims were to estimate risk of thelitis and loss of quarter symmetry or function in mid lactation, milk yield and milk solids production at first herd test and averaged over the lactation and the risk of premature culling in heifers, conditional on peripartum IMI and CM status.

Materials and methods

Herd and Heifer Selection

Thirty spring-calving dairy herds were selected for a prospective observational study on the basis that they were within a radius of 30 km of one veterinary practice (Animal

Health Centre), planned to undertake individual cow milk production testing 4 times in the lactation, used the national electronic database for recording individual animal details including breed, date of calving and milk test records, and were willing to follow the study protocol. Average size of herds from which heifers were enrolled was 332 cows (s.d. =138) and the average planned start of the calving period for the 30 herds was the 13th July 2004 (s.d. = 7 days). Average annual milk yield and milk solids (milk fat and milk protein) production for all cows was 3843 kg (s.d. = 709 kg) and 331 kg (s.d. = 53 kg), respectively; and that for first parity heifers was 3190 kg (s.d. = 648 kg) and 279 kg (s.d. = 43kg), 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 weeks before the planned starting date of the seasonal calving period. Another clinical trial was conducted concurrently in the same herds, which is to be reported elsewhere. A total of 708 heifers were enrolled in this study following consideration of prior power analysis for sample size and availability of untreated heifers from the clinical trial. 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 intramuscular antibiotics suitable 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 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 (1-2 kg of dry matter) of hay and pasture silage fed also on the grazing area.

Sample and Data Collection

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). 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 methods from all glands of each heifer within 5 days of calving during pre-planned twice weekly visits to the herds by trained technicians. If a diagnosis of CM was made before these planned visits, duplicate milk samples were taken from

all glands. Duplicate milk samples were also collected from all first cases of CM within 14 days of calving if they occurred following the pre-planned post-calving sample. Heifer data including breed, New Zealand estimated breeding value (genetic ability to transmit economically important traits especially milk production to offspring), calving date and individual animal milk production records were obtained electronically from a national database (Livestock Improvement Corporation; Hamilton, New Zealand). All individual animal disease treatments from one month before enrolment 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 all quarters of heifers treated with systemic or intramammary antibiotics in the 21 days preceding sampling were excluded from analysis. At approximately 3 months 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 non- or poorly functional mammary glands (defined as a visually-apparent smaller gland compared to the other contra-lateral gland in the same heifer immediately before attachment of milking cups).

Milk Sample Analysis

Microbiological procedures, diagnosis of IMI, and categorisation of results were undertaken using standard methodology (Hogan, et al., 1999). Milk samples were mixed thoroughly by inverting 2-3 times and then 10 µl 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 hours before reading results. All gram-positive, catalase-negative cocci were categorized as Streptococci and further speciate by CAMP test. Gram positive, catalase positive isolates were coagulase tested using a commercial kit (Staphyloslide, Becton Dickinson, USA) and categorized as either coagulase-negative Staphylococci (CNS) or coagulase-positive (assumed to be *S. aureus*). Gram negative rods that could be identified with the basic biochemical tests available to this laboratory (lactose, oxidase, TSI, LIA 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., and 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 found 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 *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.

Data Handling

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, S. agalactiae, Streptoococcus dysgalactiae* (*S. dysgalactiae*) and *S. uberis.* Minor pathogens were 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. Thus the IMI quarters also included some that were diagnosed with CM (this occurred in 195 quarters in 163 heifers). Clinical mastitis quarters were also reported 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 3. 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 (n = 46 quarters pre-calving and 55 quarters post-calving), 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 one 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 intramammary infection 'same' and intramammary infection 'new' if one of the pre-calving bacterial isolates was present at both pre- and post-calving samples (e.g. *S. aureus*) and a new bacteria was isolated post-calving (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. That is, if a heifer had one or more quarters with a major pathogen isolated as well as one or more quarters with minor pathogens isolated, it was given a status as

infected with a major pathogen. Results of heifer-level data were aggregated to the herd level to calculate prevalence of heifers within herds with IMI status prior to and within 5 days following calving, and within-herd cumulative incidence of CM within 14 days of calving. Breed of heifer was defined as the predominant parentage breed if greater than $11/16^{th}$ (Friesian or Jersey), and all other breeds including crossbreds were classified as 'other'.

Table 3. 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

Results from samples and measurements taken greater than 5 days postpartum that were for the routine postpartum sampling or greater than 14 days for CM cases were discarded for analysis. 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 from quarters that did not have a sample collected within 5 days following calving were excluded from analysis. Individual herd test records from heifers < 10 days from date of calving or within 14 days 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 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 runs within the statistical programme "R: A language and environment for statistical computing" (R Development Core Team, 2005) version 2.2.0 and obtainable from the latter's website. 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).

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. Analysis of such data with methods accounting for correlation between outcomes is necessary to avoid overly-optimistic interpretation of probability values for association and biased point estimates (Dohoo, et al., 2003, pg. 464). Hence, mixed regression models were used to account for clustering of data. Initially, unconditional associations between dichotomous outcomes and explanatory variables were examined using crude hazard ratios, incidence risk ratios and incidence rate ratios for categorical variables and univariate ordinary logistic and poisson regression for continuous variables. Unconditional associations for continuous outcome variables were examined using one-way analysis of variance. Variables with associations significant at probability values ≤ 0.2 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 *a priori* as confounders were included in the model. Possible effects of confounding were considered as shown by other variables altering the coefficients of association by > 10%, but none were found. All first order interaction terms were tested in the model in a forward stepwise manner using the same criteria.

Generalized linear mixed models were fitted with multivariate normal random effects and random intercepts by penalized quasi-likelihood for dichotomous outcomes (risk of post-calving IMI and CM, reduced quarter size or function, thelitis, ISCC > 200,000 cells/ml at herd tests and risk of premature removal from the herd) using logistic models using the "VR" package (Venables and Ripley, 2002) run within R (R Development Core Team, 2005). This package is obtainable from the latter's website,. 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 modelled using restricted maximum likelihood methods in the "nlme" package (Pinheiro, et al., 2006) run within R (R Development Core Team, 2005) and also obtainable from the latter's website. 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, and for those models with outcomes recorded at the heifer-level e.g. production and removal, 2-level models were used with herd as a random effect. Plots of residuals from final models were examined for unusual patterns, and none were found.

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:

$h_i(t|\alpha_j) = \alpha_j h_j(t) e^{\beta X}$

where α_j = frailty for j^{th} herd, $\beta X = \beta_1$ age category, and $h_i(t|\alpha_j)$ is the hazard for the i^{th} individual in the j^{th} herd at time t.

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

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

where (g) refers to the link function (logit for logistic models, and identity for linear models), Y_{ijk} is the outcome variable, β's are the model coefficients, X's are the variables included in the models (prior bacteriological status and 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: $\mu_{heifer\ (j)} \sim N(0, \sigma^2_{heifer})$, $\mu_{herd\ (k)} \sim N(0, \sigma^2_{herd})$, $\varepsilon_{ijk} \sim N(0, \sigma^2)$.

Two-level regression models may be represented as:

$(g)Y_{ij} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_n X_n + \mu_{herd\ (j)} + \varepsilon_{ij}$

Terms are as described for the 3-level model. For production outcome models, variables included were post-calving IMI and CM status, and breed, days from calving to test date, and estimated breeding value as fixed effects. Individual somatic cell counts were log-transformed for analysis, 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, as a polynomial term did not improve model fit, but a quadratic term was used for modelling average lactation ISCC.

Because outcomes measured were sometimes frequent, odds ratio measurements of association were not considered appropriate as they cannot be interpreted as multiplicative measures of risk. Instead, incidence risk ratios (RR) and incidence rate ratios (IRR) were used as they accurately describe the multiplicative incidence of an outcome occurring for a given level of exposure, compared to a reference exposure level. Incidence risk ratios and their 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). Confidence intervals for incidence rates and incidence rate ratios were calculated by exact and Wald methods, respectively. 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 was recorded in a Microsoft Access database. Statistical significance for tests was declared at $P \leq 0.05$, and confidence intervals reported are for a 95% of range of values.

Results

Heifers lost to follow-up after enrolment included 11 heifers that did not calve (9 were found to be 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 days following calving (although these heifers were present in the herds and at

risk of CM within 14 days following calving), and one heifer that was destroyed due to persistent obstetric paralysis before 14 days post-calving. Thus a total of 666 heifers were sampled within 5 days following calving, and 696 heifers were considered at risk of CM within 14 days of their calving date.

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 pre-calving was 18.5 % (CI = 17.1% to 20.0%) (Table 4). 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 =), *Klebsiella* (n = 1), *Pasteurella* spp. (n = 4) and *Proteus* spp. (n = 1). Quarter IMI prevalence pre-calving due to major pathogens was lower than that for 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. Forty of forty-seven dual infections were in combination with *S. uberis*, with a small number each of other possible combinations.

Table 4. 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 -127 to -9 days pre-calving				Prevalence of IMI 0-5 days post-calving				Cumulative incidence 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)			--	--		
Coryne. spp.[2]	7	(0.2)	1	(0.0)	2	(0.0)						
Escherichia coli	7	(0.2)	1	(0.0)	14	(0.5)	3	(0.1)	7	(3.6)	2	(1.0)
S. aureus[3]	12	(0.4)			16	(0.6)			5	(2.6)		
S. dysgalactiae[4]	2	(0.1)			10	(0.4)			6	(3.1)		
S. agalactiae[5]	1	(0.0)			--	--			--	--		
S. uberis[6]	78	(2.8)			267	(10.0)			125	(64.4)		
Other[7]	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

[2] *Corynebacterium* species

[3] *Staphylococcus aureus*

[4] *Streptococcus dysgalactiae*

[5] *Streptococcus agalactiae*

[6] *Streptococcus uberis*

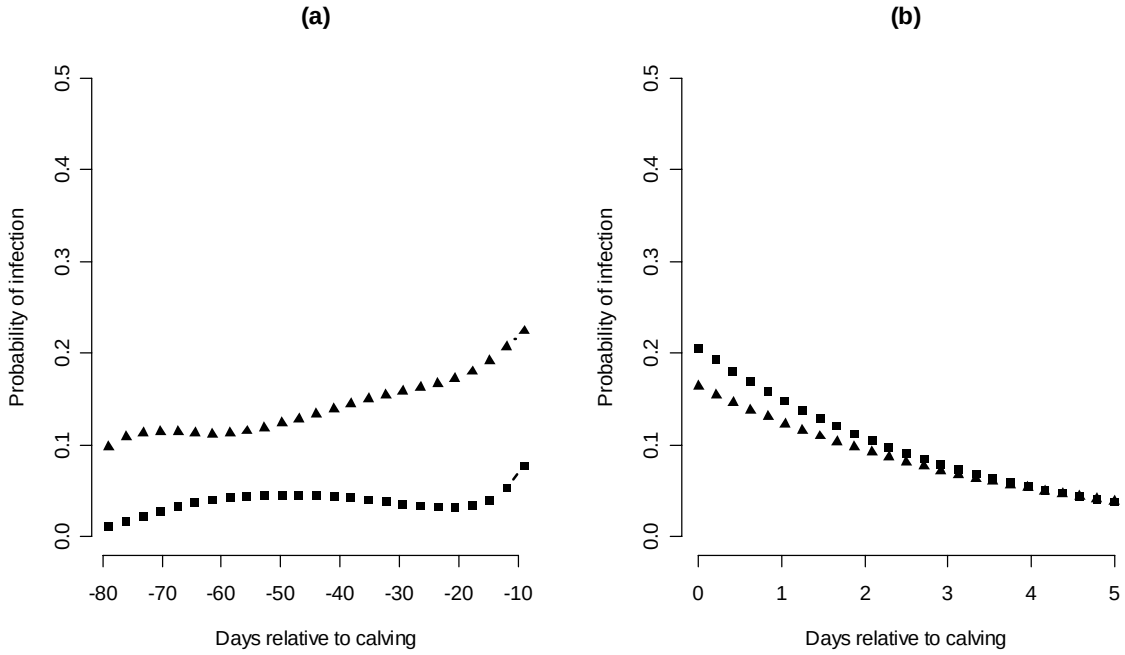
[7] *Enterococcus* spp., *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., undifferentiated gram-negative and gram-positive rods

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%). (The difference was not exactly 3.0% due to missing data from quarters with contaminated samples or those not sampled within required period not included in the paired analysis.) This change was due to an 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 1). However, the number of samples taken within 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 5^{th} 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 (a) pre-calving, and (b) post-calving, in 708 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.

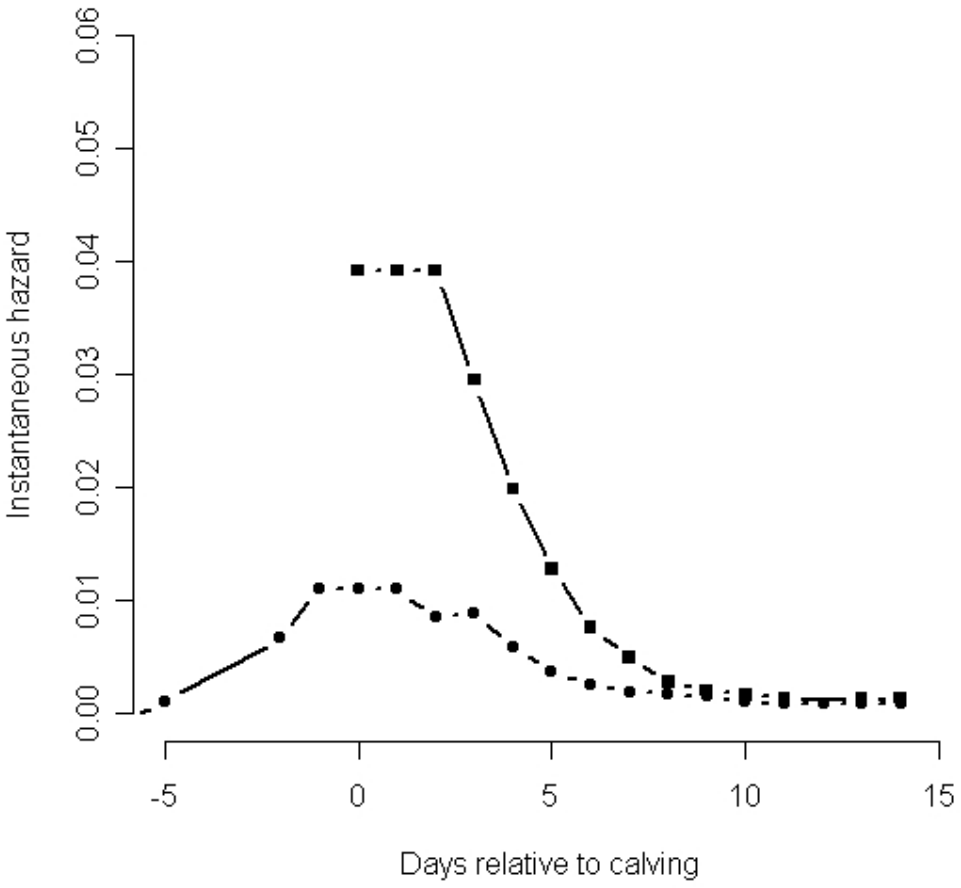
Pre-calving, 16.9% of front quarters were diagnosed with IMI, compared with 20.0% of rear quarters (difference = 3.1%, CI of difference = 0.2% to 6%). This was due to a higher proportion of major pathogen IMI in rear versus front quarters (4.7% vs. 3.1%, difference = 1.6%, CI for difference = 0.2% to 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 period of high risk 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%).

Productivity effects

Associations between post-calving IMI and clinical mastitis and diagnosis of quarters with "reduced function" or thelitis at 3 months following the end of the seasonal calving period are shown in Table 5. Ten quarters were completely non-functional, and these were included with reduced function quarters for analysis because of their low incidence. Fifteen percent of quarters with major pathogens isolated post-calving and 19% of quarters diagnosed with CM within 14 days following calving were diagnosed with reduced function (incidence risk ratios 9.4 and 9.1 compared to major pathogen-negative or CM-negative quarters, respectively). The risk of thelitis was only significantly higher in quarters with a major pathogen isolated post-calving (incidence risk ratio = 2.3), but the overall incidence of this disorder was low (< 2% of teats).

Table 5. Associations between post-calving intra-mammary infection (IMI) type and clinical mastitis (CM) and reduced quarter function and teat thelitis in mid-lactation

			Reduced function			Thelitis		
Exposure Variable	Exposed[1]	Record count	IR[2]	IRR[3]	IRR CI[4]	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	2355	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	2316	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	2055	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	2552	0.021			0.015		

[1] 1 = exposed to risk, 0 = not exposed to risk

[2] IR = incidence risk

[3] IRR = incidence risk ratio

[4] IRR CI = incidence risk ratio confidence interval

Milk yield at first herd test and average daily milk yield over the entire lactation were on average 0.6 and 0.7 kg, respectively, higher in heifers with IMI due to minor pathogens post-calving, compared to heifers with no pathogens isolated, after adjustment for effects of breed, day of lactation at test date and genetic breeding worth (Table 6). Other measures of milk yield and milk solids production were not significantly associated with post-calving bacteriological or clinical mastitis status. Isolation of major pathogens or any pathogens post-calving in one or more quarters, or clinical mastitis within 14 days of calving were significantly associated with geometric mean first herd test somatic cell counts, equivalent to increases of 22 x 10^3, 17 x 10^3 and 12 x 10^3cells/ml, respectively. Isolation of minor pathogens post-calving from one or more quarters was not significantly associated with ISCC. Culture of major pathogens from one or more quarters post-calving was associated with an increased risk of heifer ISCC > 200 x 10^3 cells/ml at Test 1 (incidence risk ratio = 2.40, CI = 1.28 to 4.48), Test 2 (incidence risk ratio = 2.86, CI = 1.63 to 5.03), Test 3 (incidence risk ratio = 2.96, CI = 1.67 to 5.25), and Test 4 (incidence risk ratio = 1.37, CI = 1.01 to 1.87). Diagnosis of CM in one or more quarters within 14 days of calving was associated with an increased risk of heifer ISCC > 200 x 10^3 cells/ml at Test 1 only (incidence risk ratio = 2.36, CI = 1.32 to 4.21). Culture of minor pathogens post-calving from one or more quarters was not associated with risk of heifer ISCC > 200 x 10^3 cells/ml at any subsequent herd test.

Table 6. Predicted population average milk volume, milk solids production and somatic cell count in heifers of differing post-calving bacteriological and clinical mastitis status

	Exposure level[1]	Milk volume		Milk solids		ISCC[2] (x 10^3)	
		Record count	Mean	Record count	Mean	Record count	Mean[3]
Exposure variable							
First herd test in lactation							
Minor pathogen	0	514	17.59	514	1.36	501	55.27
	1	94	18.18*	94	1.39	90	53.86
Major pathogen	0	405	17.68	405	1.37	398	48.89
	1	203	17.64	203	1.35	193	70.46**
Any pathogen	0	311	17.52	311	1.36	308	47.86
	1	297	17.80	297	1.36	283	65.23**
Clinical mastitis	0	481	17.63	481	1.36	473	53.88
	1	148	17.80	148	1.38	139	66.05*
Lactation Average							
Minor pathogen	0	2013	13.54	2013	1.09	1996	49.98
	1	378	14.18*	378	1.13	373	50.58
Major pathogen	0	1585	13.66	1585	1.10	1576	45.04
	1	806	13.52	805	1.08	793	61.18**
Any pathogen	0	1207	13.52	1207	1.10	1203	43.85
	1	1184	13.70	1183	1.10	1166	57.95**
Clinical mastitis	0	1896	13.62	1896	1.09	1885	48.96
	1	586	13.64	585	1.10	575	55.60*

[1] 0 = not exposed to risk, 1 = exposed to risk

[2] Individual somatic cell count

[3] Geometric mean

* $P \leq 0.05$, ** $P \leq 0.01$ for differences between exposure levels

During the entire lactation, 87 heifers (12%) were removed from the herds. The most common primary reason given for removal was failure to conceive following the seasonal breeding program (n = 65). Mastitis was given as a primary reason for removal for only 6 heifers (< 1%). Isolation of major pathogens from one or more quarters of heifers post-calving significantly increased the risk of premature culling from the dairy herd (incidence risk ratio = 1.6, CI = 1.1 to 2.3). Neither clinical mastitis (incidence risk

ratio 1.4 and CI = 1.0 to 2.1) nor isolation of minor pathogens in one or more quarters post-calving (incidence risk ratio = 0.8 and CI = 0.5 to 1.4) were significantly associated with risk of premature removal from the dairy herd.

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, prevalence of CNS at the quarter level was highest pre-calving (13.5%), and declined as lactation commenced (9.7%). Reported quarter prevalence estimates for CNS were 16% to 14% to 6% (Oliver and Sordillo, 1988); 27% to 17.8% 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. (1990a), but similar to the 0.5% reported by Aarestrup and Jensen (1997). Possible reasons for this difference include the low population of insect vectors such as flies in the Waikato region to transmit *S. aureus* (Fox, et al., 1995) and differences in housing and cow-heifer contact patterns. The pre-calving quarter level prevalence of IMI for all pathogens in this study of 18.5% of quarters is lower than that of other previously cited authors (Fox, et al., 1995, Oliver and Mitchell, 1983, Trinidad, et al., 1990a). Another feature of results from this study was the low prevalence of coliform and mixed IMI compared to other studies. Less than 0.5% of 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 faecal 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. *Sreptococcus 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* quarter level prevalence increased fourfold in this study to 10%, compared to other 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 cited 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 pasture grazing systems common 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 prevalence 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 would appear to be 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 proximity to date of 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 IMI prevalence immediately post-calving. A proportion of these subclinical

IMI may have persisted into lactation, as the higher risk of ISCC > 200,000 following major pathogen IMI suggested, but this was not confirmed by bacteriological culture.

The cumulative incidence of peripartum CM in this study also differed from those described in other countries and management systems. No pre-calving CM was diagnosed in this study, unlike that of Trinidad et al. (1990a), 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 herds in the same region as 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 separately 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 three 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 only invade glands with open teat canals. In contrast, major environmental pathogens (principally *S. uberis*) preferentially infected hind quarters of heifers in this study. This may be because transmission to these quarters is greater due to increased exposure to faecal or mud contamination through their closer proximity to the ground; higher probability of teat canal penetration; or a combination of both factors.

Isolation of *S. aureus* from quarter secretions from several weeks prior to calving shows that nulliparous pasture-grazed dairy heifers are a potential source of new infection for this pathogen to dairy herds. *Staphylococcos aureus* is usually considered to be a contagious pathogen spread between cows in lactation during the milking process. But this classification does not fit the pattern of infection of heifers in this study before the first milking. Other authors have drawn attention to the risk of introduction by heifers of this *S. aureus* to dairy herds (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 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, which is much lower than the 97% found by Trinidad et al. (1990a), 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 IMI due to major pathogens was 2.2 times 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.

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 post-calving in this study increased the risk nine-fold of that same quarter having reduced function subsequently. This agrees with the finding of Waage et al. (2000) who found 25% of quarters with CM, subsequently had one or more non-functional quarters (19% in this study), although a feature of many cases in their study was infection with *Arcanobacter spp.* and *S. aureus*, Both of these are known to cause chronic and sometimes severely damaging infections, but which were absent or rare, respectively, in this study. Similar also to their findings, thelitis occurred with higher prevalence in quarters with a major pathogen isolated post-calving. Although quarters with reduced function, asymmetry or thelitis did not have direct economic consequences measured in this study, these quarters are unsightly and may increase the difficulty of managing affected heifers because milking clusters will not hang evenly from the teats and may reduce sale value of heifers as milking animals.

Estimation of the effects of IMI and CM on milk production is not straight forward. A central problem is the confounding effect of milk production on risk of mastitis (Grohn, et al., 2004, Rajala-Schultz, et al., 1999). Cows with higher milk production potential may be at higher risk of mastitis than lower producing cows, and 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; when in fact the true loss is the difference between the (higher) unmeasured potential production and what was actually recorded. 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. But 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 both the first herd test and averaged over the total lactation were used as outcome measures to estimate effect of IMI or CM on productivity. However, the frequency of testing in N.Z herds is usually low (up to 4 tests per lactation), meaning that estimates of production are not likely to be precise, and statistically significant associations may not be detected. Further, only estimated breeding value for production traits was used in statistical models in this study to try to control the potential confounding of associations as previously discussed. It is unknown how successful this approach has been, and the possibility of uncontrolled confounding on the results still exists. Data from this study found a small but significant positive association between IMI due to a minor pathogen 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 increases milk production, but is more likely to support the finding that heifers with IMI tend to have higher milk production potential (Grohn, et al., 2004, found this tendency with CM). The same authors also showed relatively small and short-term effects of CM where *S. uberis* was isolated, which would support the finding of this study of no significant decrease in production in heifers with CM and IMI, where this was the predominant major pathogen. There are several other direct costs of mastitis, apart from production loss, which are also required to calculate the total cost of disease, including costs of therapy, milk discard, and extra labour, but these were not considered in this study.

Although CM and IMI with a major or any pathogen in early postpartum heifers in this study was significantly associated with increased mean ISCC at first herd test or averaged over the lactation, the increase over a whole season was small. However, significant associations existed between heifers with post-calving IMI due to major pathogens and heifers with CM and ISCC categorized as elevated (> 200 x 10^3 cells/ml). Of interest, is the finding that there was significant risk of high ISCC at only the first test following CM cases, whilst all tests following IMI with major pathogens

post-calving were at increased risk of having elevated ISCC. This is similar to the finding of Myllys and Rautala (1995) who found significantly increased somatic cell count only at the first herd test following CM cases, and not at subsequent tests in the lactation. This suggests that CM may have short-term effects on ISCC in heifers treated with systemic antibiotics in this study, 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. For this reason, the finding that heifers with major pathogens isolated within 5 days of calving had a 60% increased risk of removal from the herd over their lactation is important. In this study, IMI with major pathogens was diagnosed in one-third of heifers; hence the population impact of this infection on culling is likely to be high. A possible biological reason for this finding is that clinical mastitis in the postpartum period has been associated with poorer subsequent reproductive performance (Chebel, et al., 2004) via the pathway of pregnancy loss.

Errors in measurement or classification of variables is a problem in observational studies of this type (Dohoo, et al., 2003, pg. 220). Because farmers and their staff were not uniformly trained in diagnosis of CM, and classification of clinical mastitis is subjective, misclassification bias of CM was likely but its magnitude unknown. A bacteriological result of "no growth" was recorded for 18.6% of milk samples from cows diagnosed with CM, but this might not be regarded as unusually high compared to that reported in scientific literature. Reasons for "no growth" results may include low number of bacterial colonies following self-elimination, failure of sample handling and cultural methods to allow multiplication of an otherwise adequate number of bacterial colonies, or error in diagnosis of CM. Morin and Constable (1998) found that many of these "no growth" cases shared characteristics with those of Gram-negative bacterial infections. Hence it is possible that there was underreporting of gram-negative infections in this study, by an unknown but probably small amount given the low overall prevalence of these infections and unremarkable "no growth" prevalence. Because post-calving sampling was carried out twice a week on each study farm, and heifers may not have been milked until the day after calving, individual heifers were

sampled between 0 and 5 days following their calving. Further, because post-calving IMI prevalence was strongly influenced by day of sampling post-calving, it is likely that many IMI present on the day of calving had been self-eliminated within the following 5 days. Hence, prevalence for day 0-5 IMI is an underestimate of that on the day of calving. However, resources were unavailable to undertake daily sampling in each herd. Accurate determination of the status of quarter IMI over time requires genetic analysis of isolates to determine whether the same species isolated on subsequent occasions is of the same genotype. This was not undertaken at the time of preparing this dissertation; hence inferences about persistence, self-elimination or new infection status were limited to phenotypic analyses as reported here. However, where genotyping of IMI from repeated samples from the same quarter both pre- and post-calving has been done, persistence of the same infection has been reported for 9 of 12 quarters infected with *S. dysgalactiae* (Aarestrup and Jensen, 1997), and Jayarao et al. (1999) reported that most cases of CM in early lactation were preceded by subclinical infection with the same genotype.

This study is the first reported to concentrate on the epidemiology of environmental mastitis, particularly that caused by *S. uberis*, in pasture-grazed dairy heifers prior to and immediately following parturition. It provides information on patterns of IMI in heifers grazed under these management systems as commonly used in New Zealand. This information provides a basis for preventive programmes for heifers managed under these systems.

Conclusions

Results from this study show that patterns of bacterial species involved and prevalence of IMI in pasture-grazed peripartum heifers differ from those in other production systems, that the cumulative incidence of CM is high in itself and when compared to older cows. In this study, pre-calving IMI was mainly caused by the skin-opportunistic bacteria, CNS, and the quarter prevalence was relatively low. The incidence rate of new IMI immediately pre-calving by the environmental bacteria *S. uberis* was relatively high, and was highest in quarters with a minor pathogen isolated. 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 *S. 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 has not been consistently reported, but because of the preponderance of one bacterial species (*S. uberis*), effective preventive measures against this one organism e.g. by vaccination, 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.

Chapter 3- Risk Factors for Peripartum Mastitis in Pasture-Grazed Dairy Heifers

Introduction

Mastitis in dairy cows is common and is likely to impose significant economic costs on producers. International surveys of dairy herds show the incidence of clinical mastitis to be among the highest of reported diseases. Population-based surveys of clinical disease in New Zealand dairy herds are few, but available studies agree with overseas literature on the high relative incidence of mastitis. Xu and Burton (2003) reported overall CM incidence of 12% per season (compared to lameness, 5.2%; calving difficulty, 3.3%; and metabolic disease, 1.8%) and data from 38 commercial herds in early lactation reported an average cumulative incidence of clinical mastitis (CM) of 9.9% (McDougall, 1999). A review of the economics of mastitis and its control by Schepers and Dijkuizen (1991) highlighted the variability in published estimates of the costs of CM depending on assumptions and methodology, but they were in the range of U.S. $100 to $300 dollars per cow per lactation averaged over the total herd. A recent review of the costs of common diseases of dairy cows to the U.K. industry reported costs of mastitis to be approximately 3 times that of the next most costly disease (lameness). 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 approximately 10% of the total yield of the first lactation (Barnouin and Chassagne, 2001, Owens, et al., 1991).

Studies report different patterns of infection in heifers compared to older cows. Work by 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., 2004) found higher cumulative risks for Parity 1 cows compared to Parities 2 and 3 in the first 2-3 weeks of lactation, but there was variability between countries.

Important risk factors for mastitis in dairy cows depend on the pathogens involved. Increased rates of new IMI due to contagious pathogens (e.g. *S. aureus* and *S. dysgalactiae*) may be caused by persistent IMI from the previous lactation due to failure to effectively treat with non-lactating intramammary antibiotics at the last milking of the previous lactation; transmission of pathogens via milking cup liners due to inadequate machine function, poor milking technique, inadequate teat sanitization (Bramley and Dodd, 1984), and presence of teat lesions (Agger and Willeberg, 1986). However, control of pathogens of environmental origin (e.g. *S. uberis* and *E. coli*) using the same measures has not been as successful (Bramley, 1984), and researchers have more recently directed attention towards these pathogens. Important risk factors for IMI from these organisms include failure to form an effective teat plug, presence of teat end lesions, increased rates of new IMI during the dry period, increased exposure to contamination from bedding, wet udder milking preparation and milk leakage (Barkema, et al., 1999, Hillerton and Berry, 2003, Schukken, et al., 1991).

Knowledge that the pattern of CM and 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. Factors at the quarter and heifer level found to increase the risk of peripartum CM and IMI in international studies include Friesian breed (Myllys and Rautala, 1995), age at calving (de Vliegher, et al., 2004, Waage, et al., 1998) (although the effect was inconsistent between authors), dystocia (Barnouin and Chassagne, 2001, Oltenacu and Ekesbo, 1994), increased milk-out speed (Slettbakk, et al., 1990), milk leakage at calving (Waage, et al., 2001, Waage, et al., 1998), teat and udder oedema (Slettbakk, et al., 1995, Waage, et al., 2001), blood in milk (Waage, et al., 2001), decreasing teat end to floor distance (Slettbakk, et al., 1995), and pre-calving quarter IMI (Aarestrup and Jensen, 1997, Myllys, 1995). Recent research has also suggested associations between physiological changes in cows in the peripartum period, especially high serum ketone levels and negative energy balance, and decreased immune function affecting susceptibility to IMI (Kornalijnslijper, et al., 2003, Suriyasathaporn, et al., 2000). Herd-level risk factors associated with CM include high herd milk production (Myllys and Rautala, 1995, Waage, et al., 1998), high CM incidence rate (Myllys and Rautala, 1995, Waage, et al., 1998), low bulk tank milk somatic cell count (Myllys and

Rautala, 1995, Waage, et al., 1998), and small herd size (Waage, et al., 1998). However, the seasonal calving and pasture-grazing 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. This may be reflected in the relative high prevalence of *S. uberis* of all IMI in New Zealand herds (McDougall, 1998) compared to other dairy production systems.

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 have been suggested (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 (Oliver, et al., 2004, Owens, et al., 2001, Trinidad, et al., 1990b) with high rates of efficacy reported, but problems exist with prevention of antibiotic residues entering human food supply.

The main aim 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.

Materials and methods

Study Population, Data Collection and Variable Definition

This was a longitudinal observational study in the same closed population as that described in Chapter 2. Details of the study methods including microbiological techniques are also the same as those described in Chapter 2. Additionally at the time of enrolment, duplicate blood samples were collected from the caudal vein into evacuated plain glass blood collection tubes (Vacuatainer, Becton Dickinson, Franklin Lakes, USA), stored at room temperature overnight, centrifuged at 3000 RPM for 10 minutes, then serum pipetted into 5 ml factory-clean polystyrene vials and stored at minus 20° C

until processing. Visual assessment was made of the degree of udder contamination using the hygiene scoring system of 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 was assessed using the system commonly employed in N.Z of Macdonald and Roche (2004) recorded on a 1-10 ordinal scale with half score increments. The length of the tail of each heifer was recorded on an ordinal scale 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 milk sampling as described in Chapter 2, and for recording of additional measures. Enrolled heifers were visited twice-weekly by the same team of technicians for collection of duplicate individual quarter milk samples and duplicate blood samples within 5 days of their calving date, and recording of udder hygiene and body condition score. Additional measures recorded at routine post-calving visits were presence or absence of skin lesions on the barrel or orifice of each teat, minimum height of the front quarters above the floor of the milking parlour measured in centimetres using a flexible tape, presence or absence of udder oedema defined as presence or absence of a depression being present > 5 seconds after a digital depression was created in the middle of the rear of the udder, and abnormal placement or direction of teats defined as grossly abnormal position on udder or direction > 30^{o} beyond vertical. Additional data on the breed defined as > $11/16^{ths}$ Friesian or Jersey, or "other" (mainly Friesian-Jersey crossbreds), breeding worth (genetic ability to transmit economically important traits especially milk production), calving date and herd test milk production records from the current lactation were obtained electronically from Livestock Improvement Corporation (Hamilton, New Zealand). All individual animal disease treatments (including those from other parity groups) from one month before enrolment date, together with reason for removal of any subjects from the herd throughout the lactation, were collected from farm records. 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 functionality of all quarters. Quarters of same size as the contra lateral quarter were assigned a score of 0, quarters smaller than the contra lateral quarter were scored 1, and quarters not being milked because of complete loss of function were scored as 2. The

teats were also palpated for evidence of thelitis diagnosed as thickening or hardening of the wall of the teat canal. Frozen serum samples were thawed at room temperature and analyzed for concentration of beta-hydroxy butyrate (BOH) and non-esterified fatty acids (NEFA) by Gribbles Alpha Veterinary Laboratory (Hamilton, New Zealand), using a Hitachi 717 at a temperature of 30 ^{o}C. Laboratory interassay coefficient of variation (CV) for that instrument for BOH was 3%, and for NEFA was 5%. Intra-assay CV for both analyses was 1%. Definitions of the variables considered for path analysis are shown in Table 7.

Table 7. Abbreviations and definitions of variables used in null and final path models of risk factors for peripartum mastitis in pasture-grazed dairy heifers.

Sampling stage	Abbreviation	Definition and units	Measurement levels
3-4 wks prior start of heifer group calving	PREBACTALL	Absence or presence of any bacterial pathogen	0, 1
	BOH_PRE	Serum beta-hydroxybutyrate (mmol/l)	Continuous
	NEFA_PRE	Serum non-esterified fatty acids (mmol/l)	Continuous
	HYGIENE_PRE	Coverage of udder with dirt or feces: 1, clean; to 4, fully covered	1, 2, 3, 4
	BCS_PRE	Body condition score in half-unit increments	1 - 10
	TAIL_PRE	Tail length: 1, Docked short; 2, docked long; 3, switch trimmed; 4, untrimmed	1, 2, 3, 4
0 - 5 days post-calving	SCM0_5	Absence or presence of any subclinical bacterial pathogen at routine sampling 0-5 days post-calving	0, 1
	CM0_14	Absence or presence of clinical mastitis 0-14 days post-calving	0, 1
	BOH_POSTHI	Serum beta-hydroxybutyrate > 1.0 mmol/l	0, 1
	NEFA_POSTLO	Serum non-esterified fatty acids < 0.5 mmol/l	0, 1
	HYGIENE_POST	Coverage of udder with dirt or faeces: 1, clean; to 4, fully covered	1, 2, 3, 4
	BCS_POST	Body condition score in half-unit increments	1 - 10
	BCS_LOSSHI	Heifer lost > 0.5 condition score between visits	0, 1
	TAIL_POST	Tail length: 1, Docked short; 2, docked long; 3, switch trimmed; 4, untrimmed	1, 2, 3, 4
	LESION_POST	Absence or presence of 1 or more lesions on skin or teat orifice	0, 1
	MIN_HGTLO	Minimum height of front teat above ground < 53 cm	0, 1
	OEDEMA	Absence or presence of pitting oedema in skin of caudal udder	0, 1
----------	SAMPLE_CALVE_DAYS	Days from calving to sampling to calving (pre-calving negative, post-calving positive)	Continuous
	BREED	Friesian, Jersey (reference), Other	Categorical
	BW	Breeding worth- genetic merit to transmit productive traits	Continuous

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.

Data handling

Data from heifers treated with systemic or intramammary antibiotics for any reason in the preceding 21 days were excluded from analysis. Results of bacteriological testing of milk samples were summarized at the quarter-level and heifer level into pathogen categories (major or minor). Bacteriological results were categorized as either major or minor pathogens. Bacterial species classified as major pathogens were *Enterococcus spp.*, *E. coli, Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *S. aureus, S. agalactiae, S. dysgalactiae* and *S. uberis*. Minor pathogens were CNS, *Corynebacterium* spp., undifferentiated gram negative rods, undifferentiated gram positive rods and yeast. Quarters from which three of more bacterial species were isolated were defined as contaminated. Quarters with major pathogens isolated were given that status regardless of whether or not minor pathogens were also isolated from that quarter. If only minor pathogens were isolated, then that became the status.

Data analysis

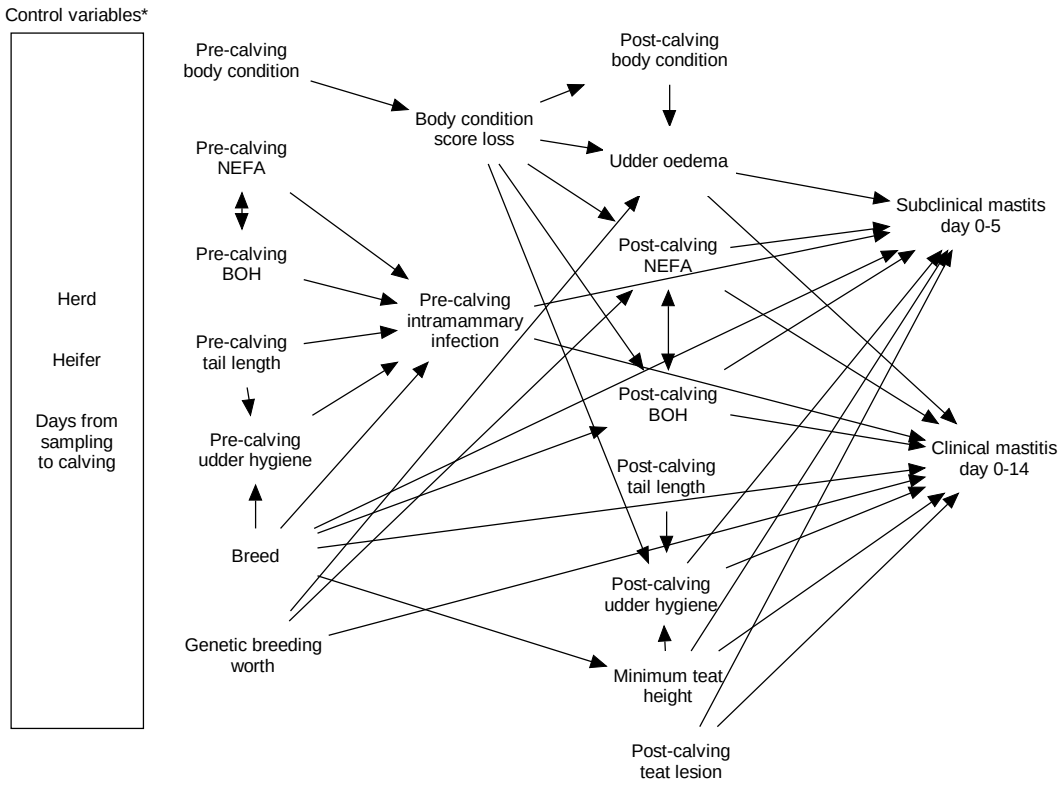
Descriptive univariate analysis was conducted on the putative risk factors and measures of IMI and CM at quarter and heifer level. Cut-points for categorization of continuous and categorical measures for use in the subsequent path analysis were determined by visual inspection of smoothed logistic regression plots (Bowman, et al., 2005) and consideration of incidence risk ratios for crude associations, respectively. 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.

Many diseases of animals are multi-factorial. These factors may be interrelated and operate in sequence or at various points in a causal pathway for that disease. 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 (Correa, et al., 1993, Erb, et al., 1985, Heuer, et al., 2001). Path analysis methods are particularly appropriate for this study because of the temporal ordering of sampling and measurements.

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 postulated (null) path model (Figure 3) 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. Variables were connected with arrows that represent hypothesised correlation (double-headed arrows) or causation (single-headed arrow from risk factor to effect). The path model is read from left to right in the direction of causation and time-order. Feedback loops were not possible as disease could only occur once and the time order is from left to right. "Exogenous" variables e.g. breed are shown with no arrows leading to them and explanations for these were not sought in the study. Control variables (herd, heifer, days between sampling and calving) were placed in a box on the far left and included in all regression models to adjust for their confounding effects. Other variables are termed "endogenous", and the relationships between these were examined in the path analysis.

Figure 3. Null path model of postulated causal pathways between measured risk factors and subclinical and clinical mastitis in 708 pasture-grazed dairy heifers.



* Variables included in regression models to control for their confounding effects

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. Correlation between outcomes needs to be accounted for in the analysis to avoid overly-optimistic interpretation of P-values for associations and biased point estimates (Dohoo, et al., 2003, pg. 464). 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:

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

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 was 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: $\mu_{heifer\ (j)} \sim N(0, \sigma^2_{heifer})$, $\mu_{herd\ (k)} \sim N(0, \sigma^2_{herd})$, $\varepsilon_{ijk} \sim N(0, \sigma^2)$.

While heifers were selected at random within farm, this approach also assumed that the 30 study farms were a reasonable representation of the population of farms.

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). Initially, unconditional (univariate) associations between outcome and explanatory variables were examined using crude risk ratios for categorical variables and univariate ordinary logistic regression for continuous variables. Statistical testing of the null path models was done using multivariable mixed binomial logistic regression models fitted by penalized quasi-likelihood with random intercepts using the bundled "VR" add-in package (Venables and Ripley, 2002) within the statistical software "R" (R Development Core Team, 2005). Each endogenous variable was regressed on all antecedent variables directly linked to it by hypothesized causal pathways in a backward stepwise method. Wald tests were used to assess the significance of terms in the model, and those with values ≤ 0.05 were included. Confounding variables altering the coefficients of association > 10% were retained in the model even if not significant. First order interaction terms were tested in the model in a forward stepwise manner using the same criteria. Finally, control variables were forced into each model. Model residuals were inspected for unusual patterns. Data from a small number of measurements and samples were missing (maximum = 6%, see Table 2) therefore the sample size varied slightly between models. 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. Mathematically, it is expressed as:

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

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 (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 ≤ 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

A full description of the microbiological results by bacteria species is reported in Chapter 2. In summary, pre-calving prevalence of quarters with any pathogen was 18.5%. Coagulase negative staphylococci were the most prevalent pathogens isolated (13.5%), and in decreasing proportion were *S. uberis* (2.8%), other gram negative bacteria (0.5%) and *S. aureus* (0.4%). Post-calving prevalence of IMI with any pathogen was 21.5%. *Streptococcus uberis* were the most common pathogen post-calving (10.0%), and in decreasing proportion were CNS (9.7%), *S. aureus* (0.6%) and *E. coli* (0.5%). 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* (64.4% of results), with lesser percentages of CNS (7.7%), *E. coli* (3.6%), *S. dysgalactiae* (3.1%) and *S. aureus* (2.6%).

The variables used in null and final models for path analysis, with their time of sampling and descriptive statistics, are shown in Table 8. Udder hygiene of heifers pre-calving was predominantly Score 1 (55%), and Score 2 (27%). Post-calving, Score 2 udders were more prevalent (38%) and "clean" (Score 1) udders had declined in prevalence to 37%. For paired records, udder hygiene score 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 ($P < 0.01$, in regression models). Teat-end lesions were recorded in only 33 (1%) of quarters within 5 days following calving and they were not significantly associated with either SCM0_5 or CM0_14. Teat position was scored as abnormal in only 11 quarters (0.3%), and was not considered in the analysis because of its very low prevalence. Tails of heifers pre-calving were mostly of their natural length (81%), and only 4% had been docked short. Within 5 days of calving, almost two-thirds of tails had been trimmed (score 3), and 10% had been docked short. Tail-length was not found to be associated with udder hygiene score either pre- or post-calving. Median body condition score pre-calving of heifers was score 6 (range 4.5 to 8.0). Post-calving median condition score declined to 5.0, (range 3.5 to 6.5). Serum BOH concentrations pre-calving had a median of 0.7 mmol/l, but 21% of heifers had concentrations > 1.0 mmol/l. Serum NEFA concentrations at the same sampling time 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 mmol/l and 0.94 mmol/l, respectively.

Table 8. Description of variables used in null and final path models of risk factors for peripartum mastitis in pasture-grazed dairy heifers.

Continuous Measures

Variable	Min.	1st Quartile	Median	3rd Quartile	Max.	Number records
Pre-calving						
BOH_PRE (mmol/l)	0.30	0.60	0.70	0.90	4.70	708
NEFA_PRE (mmol/l)	0.01	0.26	0.39	0.56	2.38	708
BCS_PRE	4.5	5.5	6	6.5	7	708
SAMPLE_CALVE_DAYS	-9	-30	-39	-50	-127	697
Post-calving						
[1]BOH_POST (mmol/l)	0.10	0.60	0.70	0.90	2.70	672
[1]NEFA_POST (mmol/l)	0.07	0.42	0.64	0.94	2.18	672
BCS_POST	3.5	5	5	5.5	6.5	662
[1]BCS_CHANGE	-2.5	-1.5	-1	-0.5	1	662
[1]MIN_HGT (cm)	39	49	52	55	66	654
SAMPLE_CALVE_DAYS	0	1	2	3	5	665

Categorical Measures	Proportion in each category				Number records
Pre-calving	1	2	3	4	
TAIL_PRE	0.038	0.027	0.126	0.809	708
HYGIENE_PRE	0.551	0.274	0.088	0.087	704
Post-calving					
TAIL_POST	0.097	0.009	0.627	0.267	663
[1]HYGIENE_POST	0.366	0.385	0.169	0.080	662
	Friesian	Jersey	Other		
BREED	0.411	0.302	0.287		708

Dichotomous Measures	Level	No. cases	Denom-inator	Incidence
PREBACTALL	Qtr.	502	2710	0.185
BOH_POSTHI	Hfr.	121	672	0.180
NEFA_POSTLO	Hfr.	223	672	0.332
MIN_HGTLO	Hfr.	361	654	0.552
OEDEMA	Hfr.	401	659	0.609
LESION_POST	Qtr.	32	2630	0.012
BCS_LOSSHI	Hfr.	118	662	0.178
HYGIENE_POSTPOOR	Hfr	420	662	0.634
POSTBACTALL	Qtr.	569	2644	0.215
CM0_14	Qtr.	195	2788	0.070
SCM0_5	Qtr.	473	2644	0.179

[1]Variables used in dichotomous form in final regression models

Direct associations (estimated as relative risk from regression analysis of variables in the final model) are shown in Table 9 and these are used in the final path model (Figure 4). The associations of pre-calving IMI (PREBACTALL) with SCM0_5 and CM0-14

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- NEFA_POSTLO was associated with a relative increase in the risk of CM of 60% (i.e. 60% more cases of CM), and BOH_POSTHI increased the risk of OEDEMA by 20%. Risk of oedema was increased 20% by heifers who lost > 0.5 BCS units compared to those which lost ≤0.5 BCS. Post-calving hygiene scores > 1 increased the risk of quarters being diagnosed with SCM0_5 by 30%, and heifers with minimum teat height less than the median had 30% and 110% increased risk of being diagnosed with SCM0_5 and CM0_14, respectively.

Table 9. Description of regression models used in final path model of risk factors for peripartum mastitis in pasture-grazed dairy heifers.

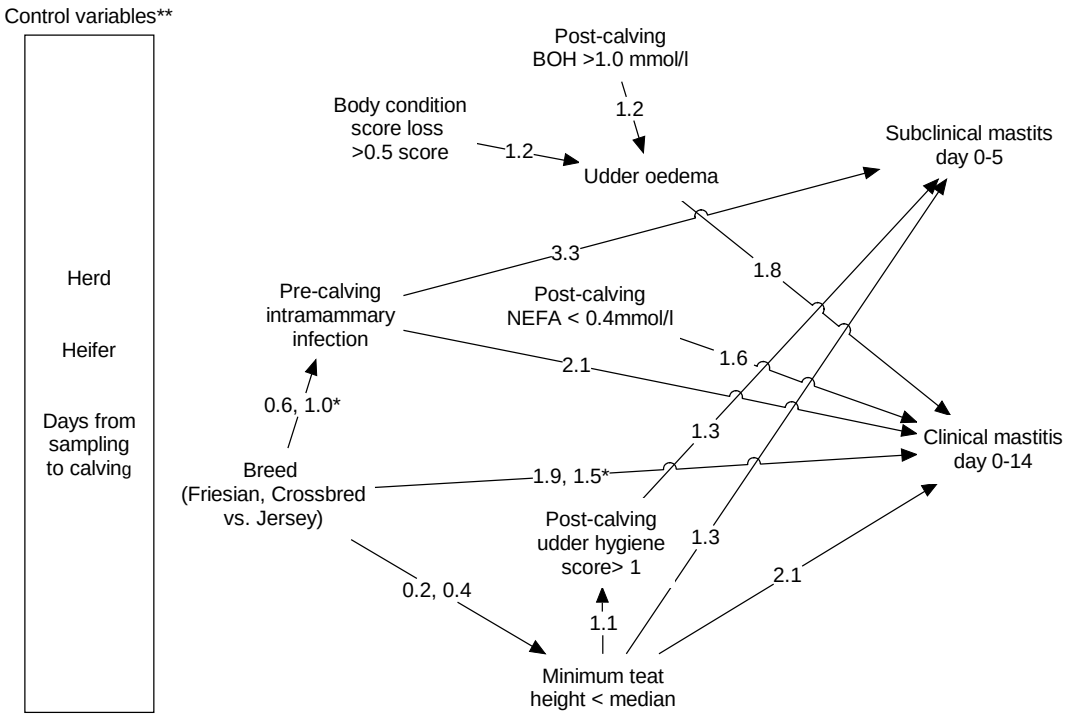
Dependent variable	Independent variables	Coeff.	Error	D.f	P-value	RR[1]	LCL[2]	UCL[3]
PREBACTALL	(Intercept)	-1.94	0.21	1970	0.00	---	---	---
	BREED FRIESIAN	-0.46	0.16	664	0.01	0.63	0.46	0.87
	BREED JERSEY	0.05	0.17	664	0.77	1.05	0.75	1.46
	SAMPLE_CALVE_DAYS	0.00	0.00	664	0.45	1.00	0.99	1.00
MIN_HGTLO	(Intercept)	2.06	0.29	631	0.00	---	---	---
	BREED FRIESIAN	-2.95	0.34	631	0.00	0.17	0.10	0.28
	BREED JERSEY	-1.70	0.32	631	0.00	0.40	0.26	0.58
HYGIENE_	(Intercept)	-0.46	0.07	621	0.00	---	---	---
POST	MIN_HGTLO	0.12	0.06	621	0.05	1.13	1.00	1.28
POOR	SAMPLE_CALVE_DAYS	-0.02	0.02	621	0.15	0.98	0.95	1.28
OEDEMA	(Intercept)	-0.80	0.08	629	0.00	---	---	---
	BREED FRIESIAN	0.30	0.08	629	0.00	1.35	1.15	1.59
	BREED JERSEY	0.18	0.09	629	0.06	1.20	1.00	1.43
	BCS_LOSSHI	0.14	0.07	629	0.03	1.16	1.01	1.32
	BOH_POSTHI	0.15	0.07	629	0.03	1.17	1.02	1.34
	SAMPLE_CALVE_DAYS	-0.11	0.02	627	0.00	0.90	0.86	0.94
SCM0_5	(Intercept)	-2.62	0.13	1829	0.00	---	---	---
	PREBACTALL	1.20	0.07	1829	0.00	3.32	2.87	3.83
	MIN_HGTLO	0.28	0.12	620	0.02	1.32	1.05	1.66
	HYGIENE_POSTPOOR	0.28	0.12	620	0.02	1.32	1.05	1.65
	SAMPLE_CALVE_DAYS	-0.17	0.03	633	0.00	0.84	0.79	0.90
CM0-14	(Intercept)	-4.90	0.30	1825	0.00	---	---	---
	PREBACTALL	0.76	0.10	1825	0.00	2.14	1.76	2.59
	NEFAPOST_LO	0.44	0.18	612	0.02	1.55	1.08	2.22
	OEDEMA	0.59	0.19	612	0.00	1.81	1.26	2.60
	BREED FRIESIAN	0.66	0.27	612	0.01	1.94	1.15	3.27
	BREED JERSEY	0.40	0.25	612	0.10	1.49	0.92	2.42
	MIN_HGTLO	0.72	0.21	612	0.00	2.05	1.36	3.08

[1] Incidence risk ratio

[2] 95% lower confidence limit

[3] 95% upper confidence limit

Figure 4. Final path model for significant risk factors[1] for subclinical and clinical mastitis in 708 pasture-grazed dairy heifers



*Coefficient for Jersey vs. Crossbred not significant

** Variables included in regression models to control for their confounding effects

Population attributable fractions for the outcomes SCM0_5 and CM0_14 are shown in Table 10. 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 10. 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	[1]LCL	[2]UCL
[3]SCM0-5	PREBACTALL	0.30	0.26	0.34
	MIN_HGTLO	0.15	0.03	0.25
	HYGIENE_POSTPOOR	0.17	0.06	0.27
[4]CM0-14	PREBACTALL	0.17	0.10	0.24
	NEFALOPOST	0.27	0.19	0.35
	OEDEMA	0.33	0.15	0.47
	BREED FRIESIAN	0.28	0.07	0.44
	MIN_HGTLO	0.37	0.16	0.53

[1] 95% lower confidence limit

[2] 95% upper confidence limit

[3] Subclinical mastitis 0-5 days post-calving

[4] Clinical mastitis diagnosed 0-14 days post-calving

Discussion

Pre-calving, the minor pathogen, CNS, was most prevalent, and the major pathogens (*S. uberis, S. aureus,* and *E. coli)* were relatively infrequent. This pattern of relative importance of CNS was similar to that found by other authors (Aarestrup and Jensen, 1997, Oliver and Sordillo, 1988). Post-calving, major pathogens greatly increased in prevalence, especially that of *S. uberis* (10% of all quarters); a prevalence similar to that found by Oliver and Sordillo (1988), but higher than that reported by Aarestrup and Jensen (1997). The cumulative incidence of clinical mastitis in this study was higher than that reported by Barnouin and Chassagne (2001), and in an earlier study in the same region by Pankey et al (1996). *Streptococcus uberis* was the most common pathogen from cases of CM, supporting earlier findings from studies of all age cows in New Zealand (McDougall, 1998, McDougall, et al., 2004). The relatively high prevalence of one major pathogen (*S. uberis*) in this study was in contrast to findings

by others (Jonsson, et al., 1991, Waage, et al., 1999a), where a wider range of pathogens were isolated. This underlines the importance of determining effective preventive measures directed specifically against *S. uberis*, such as the use of vaccines and management practices that reduce environmental exposure or enhance immunity.

Body condition is a measure of fat reserves, and it is common for cows to loose body condition in late pregnancy and in early lactation because of reduced energy intake and increased energy demand of late pregnancy and commencement of lactation (Herdt, 2000a). Excessive body condition loss results in elevated NEFA and BOH concentrations, and may have deleterious effects on future production and reproduction (Adewuyi, et al., 2005, Oetzel, 2004). Cows in positive energy balance have NEFA concentrations < 0.2 mmol/l, and concentrations > 0.7 mmol/l post-calving indicate severe negative energy balance (NEB) (Adewuyi, et al., 2005) . Subclinical ketosis was defined by Oetzel (2004) with a cut-point > 1.4 mmol/l BOH, and elevated pre-fresh (2-14 days pre-calving) NEFA concentrations as > 0.4 mmol/l. Non-esterified fatty acids are however thought to be more directly related to energy balance than BOH concentrations (Herdt, 2000b). In this study population, heifers lost a median of 1.0 body condition score units pre-calving, and it would be expected that further condition loss would occur in the following weeks of early lactation. Using these reference ranges, pre-calving, 5% of heifers had subclinical ketosis, and 50% had NEFA levels elevated above that normal for animals closer to calving, and 80% were in NEB. Post-calving, < 10% of heifers had subclinical ketosis, but 75% of heifers were in severe NEB. Together, these results show energy intake of pasture-grazed heifers in the prepartum period was mostly deficient, and often severely so.

Udder oedema was common in this study population and an important risk factor for CM. Causes of udder oedema are not clearly defined (Al-Ani and Vestweber, 1986), but associations have been made with secretion of mammary estrogens and potential milk yield (Janowski, et al., 2002). The finding that udder oedema in heifers increases CM cases by 80% is similar to the association 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 a loss of the flushing effect, allowing pathogen numbers

to increase above a threshold which overwhelm udder defence mechanisms resulting in CM. Elevated serum concentrations of BOH post-calving indicate lack of adaptation to high levels of body fat mobilization in response to inadequate dietary energy intake (Herdt, 2000a). This may 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 associated with increased risk of udder oedema and were indirectly associated with CM 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. Body condition scores were recorded at varying intervals between pre- and post-calving visits, and it cannot be assumed that the rate or timing of fat mobilization relative to calving was the same for animals with the same body condition score change; hence there was the possibility of measurement error and failure to find associations actually present. Although it is plausible that heifers which lost large amounts of body condition might also have elevated BOH concentrations, this association was not found in the data, possibly for reasons just given.

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 any prior infection. Data from this study found a significant increase in udder contamination between pre and post-calving measurement. This suggests heifer management factors or behaviour may differ between these periods, and that increased exposure of udders to environmental pathogens was associated with increased risk of environmental IMI. 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. Tail length of heifers was not found to be associated with udder hygiene, in agreement with the finding of Schreiner and Ruegg (2002) in multiparous dairy cows. This reinforces the view that tail-docking to improve udder hygiene and health is not supported by scientific evidence.

The presence of teat-end lesions within 5 days of calving was not associated with either SCM or CM, in contrast to the findings of Agger and Willerberg (1986) in all age cows. The low prevalence of teat-end lesions and brief period of observation in this study meant low statistical power and limited time for exposure to show a significant result. However, because of the low prevalence of teat-end lesions, this condition is unlikely to have an important population impact on peripartum IMI in heifers.

This study found increased risk of CM in heifers of Friesian breed (RR = 2.2) compared to Crossbred cattle, and despite their lower prevalence of pre-calving IMI and increased minimum teat height. 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). Friesian heifers were not however at greater risk of SCM, suggesting that immune response to IMI in this breed differs from that in others. Results from this and other studies suggest that 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 CM.

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. This association is in part due to repeat culture of the same organism, and infection with a new pathogen (Chapter 2). In cows, the presence of a keratin "plug" in the teat canal is protective against new IMI over the non-lactating period (Dingwell, et al., 2004, Smith, et al., 1985b). Prevalence of teat plugs in heifers before calving has not been reported. 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 with both major and minor pathogens in last few weeks of gestation. This would explain the observed increase in the risk of IMI and CM post partum when

pathogens were isolated from the same quarter 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 SCM0_5 (RR = 3.3) than with CM0_14 (RR = 2.1). This may be because only a small proportion of SCM0_5 actually become clinical, and that 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 from this study (Chapter 2) 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 methods showed that both low minimum height of teats above the ground and poor udder hygiene were positively associated with SCM0_5 and CM0_14. 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.

The second piece of evidence to support this view came from the unexpected finding that high NEFA concentrations post-calving were protective of CM. Research suggests 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 were not collected in this study, it is possible that heifers loosing little body condition (low NEFA post-calving) produced more milk immediately pre-calving, which required storage in the udder until the first milking, increasing intramammary and teat pressure and increasing the risk of

loss of teat canal integrity and milk leakage. Therefore POST_NEFALO may be a proxy variable for milk leakage or open teat canals pre-calving. Conversely, acute and greater body fat mobilization immediately pre-calving reduced lactogenesis, lowering the risk of milk leakage. 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 nutritional management options exist for control of CM, and that its risk may be reduced by targeting below maintenance feed intakes of heifers immediately pre-calving. Loss of ≤ 0.5 BCS is associated with less udder oedema and 'protective' concentrations of NEFA, but was not associated with decreased milk production in first or subsequent milk tests (data not shown). However, nutritional management interventions need to be field-trialled before recommendations can be made. Measures to enhance the hygiene of heifers' pre-calving environment should also be promoted, as reduced exposure to this source of pathogens is likely to reduce the risk of mastitis post-calving.

Estimates of PAF and their uncertainty as shown by width of confidence intervals may be used to guide intervention measures. CM is the primary concern of producers so effective measures directed against oedema are most likely to reduce its population incidence, although this condition is in part physiological and may be positively associated with milk production potential (Janowski, et al., 2002). Therefore, selection for high production may increase the prevalence of udder oedema and CM. 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 is possibly a higher priority in preventive strategies. Caution should be taken in generalizing PAFs to other populations because they are based on estimates from the model, only those covariates included and the exposure distribution of the sample from which they are based. However, in the absence of other data from the target population, these may serve as a guide for determining preventive measures.

There are several areas in which the epidemiology of peripartum mastitis in pasture-grazed heifers is poorly understood. New knowledge is needed on factors affecting IMI

in heifers from a month or more prepartum, as these infections are associated with the pattern of IMI and CM after calving. Risk factors such as udder oedema and milk leakage have already been identified, but management factors affecting them need to be determined so that interventions at the herd level can be applied. The results from this study do suggest that exposure to environmental bacterial pathogens increased up to the time of calving and that physiological changes in the immediate pre-calving and calving period were associated with risk of mastitis. However, these changes need to be more precisely measured and their mechanisms defined, before robust preventive recommendations can be given.

Conclusions

Data presented from this study suggest that important risk factors for post-calving SCM and CM may be grouped into those affecting host immunity and exposure to environmental intramammary pathogens. Both groups of factors are at least in part amenable to management interventions, and provide opportunities to reduce the burden of mastitis in heifers. Control of the risk factors of poor udder hygiene, udder oedema and pre-calving intramammary infection, through improved heifer management are most likely to have greatest impact on reducing post-calving IMI and CM in pasture-grazed dairy heifers.

Chapter 4- Conclusion

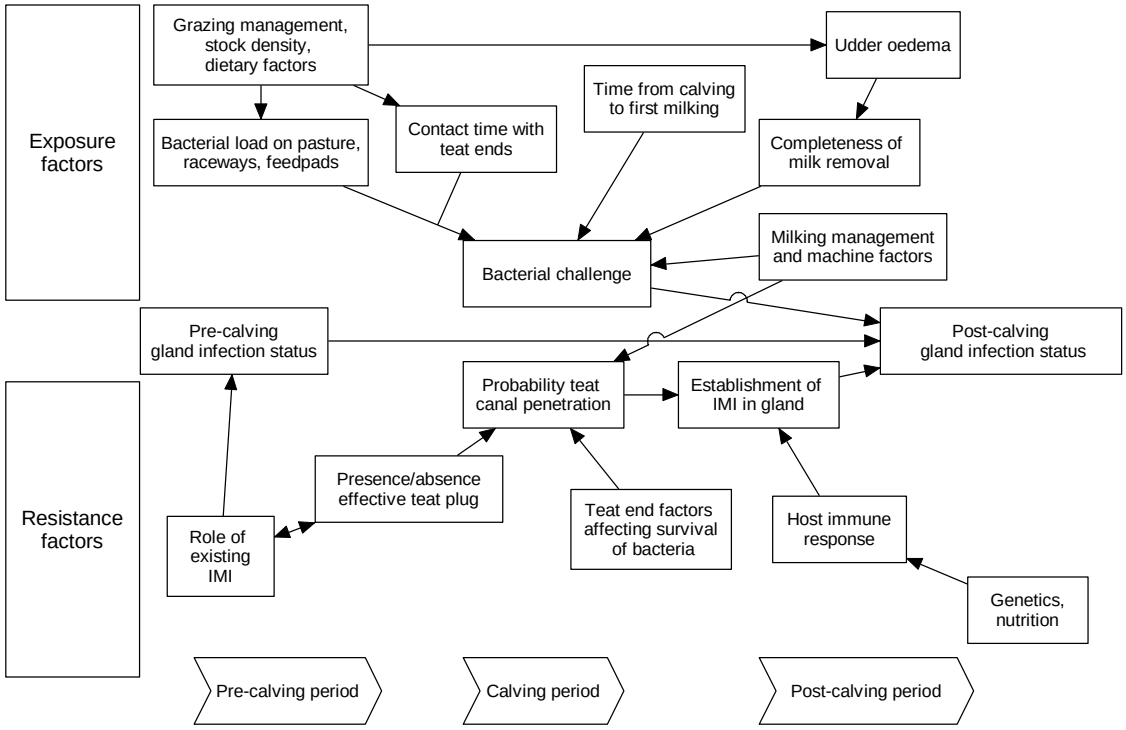
Data from this study both support findings from others and provide new insights into the epidemiology of mastitis in peripartum heifers. Intramammary infection (IMI) and clinical mastitis (CM) are common in pasture-grazed heifers as managed in New Zealand, a finding in common with other studies. Prior to calving, CNS IMI predominates, although at lower prevalence than that found in overseas studies. The finding that *S. uberis* was the most common isolate from cases of CM is also similar to that found internationally, but its relative importance in New Zealand is greater because of the low prevalence of other bacterial species e.g. *S. aureus* and *E. coli* and because it causes a high incidence rate of new infections in the immediate pre-partum period. Significant risk factors for heifer mastitis found in this study in common with others were presence of an intramammary infection prior to calving, udder oedema, low minimum teat height above the ground, and Friesian breed. However, factors not previously demonstrated in heifers as being risk factors for peripartum mastitis were associated with an increase in environmental exposure and changes in physiology- poor post-calving udder hygiene, and post-calving nutritional management indicators of low serum non-esterified-fatty acid (NEFA) concentrations, and high body condition score loss and high ketone concentrations (indirectly through the path of udder oedema). Data shows that the presence of IMI pre-calving is associated with the largest fraction of mastitis in the heifer population, but that Friesian breed and low NEFA concentrations also have high population impact. In common with some other studies, no association was found between mastitis in heifers and both milk production and increased risk of early.

Some features of the study methods have limited the findings made. Pre-calving intramammary samples were taken once only approximately 4 weeks before the planned start of calving for each herd's heifers, and therefore no reliable estimate of prevalence of IMI in the last 14-21 days of pregnancy was possible. Because this is the period of highest new infection rate, its estimation and knowledge of the factors that determine it would greatly help in targeting preventive measures at this critical time. One problem with milk sampling heifers (or cows) pre-calving is that any teat plug present will be removed along with the sample, and this may affect the local immunity of that gland.

Hence, a study design that accounts for this is required to estimate IMI prevalence pre-calving, such as random sampling of one gland within each heifer, on each of 4 separate occasions over time. However this could not be implemented because of limited resources. Increased precision of variable measurement may have improved the ability to detect associations between putative risk factors and outcomes. Measurement of udder oedema in heifers using a wider ordinal scale may have increased understanding of this important risk factor. The timing of recording of body condition score and udder hygiene relative to calving were not optimum, because they were not always close to the day of calving or when the risk factor may have impacted on new IMI rates. More frequent measurement of these variables immediately prior to calving would have enhanced accuracy. No recording of milk leakage was carried out because technicians were not on-farm every day or prior to the time of milking, so this important variable could not be assessed. Again, more frequent measurements at strategic times might have overcome this problem. Finally, data on heifer parentage or genotype might have enhanced understanding of genetic effects on mastitis.

One of the goals of research is to provide a basis to direct future studies. A diagram may be drawn of possible causal pathways involved in heifer mastitis (Figure 5) to illustrate some areas where new knowledge is required.

Figure 5. Postulated causal pathway for factors affecting peripartum mastitis in dairy heifers that require more knowledge.



New knowledge in these areas may assist in developing preventive strategies. Changes to grazing and nutritional management pre-calving offer the greatest promise to reduce pathogen load in their environment by minimising mud and faecal contamination of pastures, and the contact between pathogens and the teat ends. An indirect effect of grazing management is that it may also affect udder defence mechanisms via the pathways of milk leakage or teat plug efficacy and udder oedema. The other direct path to reduce heifer mastitis is to intervene directly with the heifer. This may be to either enhance heifer udder defence mechanisms by way of specific pathogen vaccination, or by provision of an artificial teat plug. The former is being actively researched at present, and the latter has already undergone successful randomised clinical trials (personal communication, Katrina Parker). The use of prophylactic intramammary antibiotics pre-calving has proven to be highly effective, but is unlikely to be widely used in New Zealand because of extra management requirements to mitigate the risks of antibiotic residues entering the milk or meat supply, and political opinions which question their use where other alternatives are available.

There is a long history of research into mastitis in dairy cows, but that specifically towards heifers is comparatively brief and fragmented. Rapid advances in control of mastitis in cows were not made until developments in the recognition of the role of infectious pathogens and how they might be controlled with disinfection, antibiotics, and improved milking machine function. However, control of mastitis in cows due to environmental pathogens has been slow by comparison. Hence, it may be unrealistic to expect rapid progress in controlling mastitis in heifers (mainly due to environmental pathogens). They are handled less and managed more extensively than cows in milk, as well improvements in control of environmental mastitis in lactating cows haven't even been achieved. That is not say that the goal of controlling mastitis in heifers is not attainable, because this study has shown that potential already exists for improvement and potential research directions have now been identified.

References

Aarestrup, F. M. and N. E. Jensen. 1997. Prevalence and duration of intramammary infection in Danish heifers during the peripartum period. Journal of Dairy Science. 80(2):307-312.

Adewuyi, A. A., E. Gruys, and F. J. van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Veterinary Quarterly. 27(3):117-126.

Agger, J. F. and P. Willeberg. 1986. Epidemiology of teat lesions in a dairy herd. II. Associations with subclinical mastitis. Nordisk Veterinary Medicine. 38:220-232.

Al-Ani, F. K. and J. G. E. Vestweber. 1986. Udder Oedema: An updated review. Veterinary Bulletin. 56(9):763-769.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, G. Benedictus, and A. Brand. 1999. Management practices associated with the incidence rate of clinical mastitis. Journal of Dairy Science. 82(8):1643-1654.

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. Journal of Dairy Science. 81(2):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. Canadian Veterinary Journal. 42(1):47-53.

Barto, P. B., L. J. Bush, and G. D. Adams. 1982. Feeding Milk Containing Staphylococcus-Aureus to Calves. Journal of Dairy Science. 65(2):271-274.

Bennett, R. M., K. Christiansen, and R. S. Clifton-Hadley. 1999. Estimating the costs associated with endemic diseases of dairy cattle. Journal of Dairy Research. 66(3):455-459.

Bigras-Poulin, M., A. H. Meek, S. W. Martin, and I. McMillan. 1990. Health problems in selected Ontario Holstein cows: frequency of occurrences, time to first diagnosis and associations. Preventive Veterinary Medicine. 10(1-2):79-89.

Boddie, R. L., S. C. Nickerson, W. E. Owens, and J. L. Watts. 1987. Udder microflora in nonlactating heifers. Agri-Practice. 8(2):22-25.

Bowman, A. W. and A. Azzalini. 1997. Applied Smoothing Techniques for Data Analysis: the Kernel Approach with S-Plus Illustrations. Oxford University Press, Oxford.

Bowman, A. W., A. Azzalini, and B. D. Ripley. 2005. sm: Smoothing methods for nonparametric regression and density estimation. R package version 2.1-0.

Bramley, A. J. 1984. Beecham Mastitis Series - Streptococcus-Uberis Udder Infection - a Major Barrier to Reducing Mastitis Incidence. British Veterinary Journal. 140(4):328-335.

Bramley, J. A. and F. H. Dodd. 1984. Reviews of the progress of Dairy Science: Mastitis control- progress and prospects. Journal of Dairy Research. 51:481-512.

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. Animal Reproduction Science. 84(3/4).

Cooper, M. G., B. M. Buddle, and M. G. Ashby. 1977. Incidence of infection prior to first parturition. 1976-1977 Annual Report Wallaceville, New Zealand, Animal Research Centre.1.

Correa, M. T., H. Erb, and J. Scarlett. 1993. Path-Analysis for 7 Postpartum Disorders of Holstein Cows. Journal of Dairy Science. 76(5):1305-1312.

Curtis, C. R., M. D. Salman, D. Strickland, B. Edmonston, and H. N. Erb. 1988. Path-Analysis Using Logistic-Regression - Interpretational and Methodologic Issues. Acta Veterinaria Scandinavica. Suppl. 84:469-472.

de Vliegher, S., H. W. Barkema, G. Opsomer, A. de Kruif, and L. Duchateau. 2005a. Association between somatic cell count in early lactation and culling of dairy heifers using cox frailty models. Journal of Dairy Science. 88(2):560-568.

de Vliegher, S., H. W. Barkema, H. Stryhn, G. Opsomer, and A. de Kruif. 2005b. Impact of early lactation somatic cell count in heifers on milk yield over the first lactation. Journal of Dairy Science. 88(3):938-947.

de Vliegher, S., H. Laevens, H. W. Barkema, I. R. Dohoo, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Management practices and heifer characteristics associated with early lactation somatic cell count of Belgian dairy heifers. Journal of Dairy Science. 87(4):937-947.

de Vliegher, S., H. Laevens, L. A. Devriese, G. Opsomer, J. L. Leroy, H. W. Barkema, and A. de Kruif. 2003. Prepartum teat apex colonization with Staphylococcus chromogenes in dairy heifers is associated with low somatic cell count in early lactation. Veterinary Microbiology. 92(3):245-252.

Dingwell, R. T., K. E. Leslie, Y. Schukken, J. M. Sargeant, L. L. Timms, T. E. Duffield, G. P. Keefe, D. E. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Preventive Veterinary Medicine. 63(1-2):75-89.

Dohoo, I., W. Martin, and H. Stryhn. 2003. Veterinary Epidemilogic Research. Atlantic Veterinary College Inc., Charlottetown, Canada.

Dohoo, I. R., S. W. Martin, A. H. Meek, and W. C. D. Sandals. 1983. Disease, production and culling in Holstein-Friesian cows. 1. The data. Preventive Veterinary Medicine. 1:321-334.

Dohoo, I. R., A. H. Meek, S. W. Martin, and D. A. Barnum. 1981. Use of Total and Differential Somatic-Cell Counts from Composite Milk Samples to Detect Mastitis in Individual Cows. Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee. 45(1):8-14.

Edinger, D., B.-A. Tenhagen, P. Kalbe, G. Kluender, B. Baumgaertner, and W. Heuwieser. 2000. Effect of teat dipping with a germicide barrier teat dip in late gestation on intramammary infection and clinical mastitis during the first 5 days post-partum in primiparous cows. Journal of Veterinary Medicine - Series A. 47(8):463-468.

Erb, H. N., R. D. Smith, P. A. Oltenacu, C. L. Guard, R. B. Hillman, P. A. Powers, M. C. Smith, and M. E. White. 1985. Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows. Journal of Dairy Science. 68(12):3337-3349.

Etherington, W. G., S. W. Martin, I. R. Dohoo, and W. T. K. Bosu. 1985. Interrelationships between Postpartum Events, Hormonal-Therapy, Reproductive Abnormalities and Reproductive-Performance in Dairy-Cows - a Path-Analysis. Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee. 49(3):261-267.

Fox, L. K., S. T. Chester, J. W. Hallberg, S. C. Nickerson, J. W. Pankey, and L. D. Weaver. 1995. Survey of intramammary infections in dairy heifers at breeding age and first parturition. Journal of Dairy Science. 78(7):1619-1628.

Greenland, S. 2001. Estimation of population attributable fractions from fitted incidence ratios and exposure survey data, with an application to electromagnetic fields and childhood leukemia. Biometrics. 57(1):182-188.

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. Journal of Dairy Science. 87(10):3358-3374.

Harmon, R. J. and B. E. Langlois. 1989. Mastitis Due to Coagulase-Negative Staphylococcus Species. Agri-Practice. 10(1):29-34.

Herdt, T. H. 2000a. Ruminant Adaptation to Negative Energy Balance: Influences on the Etiology of Ketosis and Fatty Liver. Veterinary Clinics of North America: Food Animal Practice. 16(2):215-230.

Herdt, T. H. 2000b. Variability Characteristics and Test Selection in Herd-Level Nutritional and Metabolic Profile Testing. Veterinary Clinics of North America: Food Animal Practice. 16(2):387-403.

Heuer, C., Y. H. Schukken, L. J. Jonker, J. I. D. Wilkinson, and J. P. T. M. Noordhuizen. 2001. Effect of monensin on blood ketone bodies, incidence and recurrence of disease and fertility in dairy cows. Journal of Dairy Science. 84(5):1085-1097.

Hillerton, J. E. and E. A. Berry. 2003. The management and treatment of environmental streptococcal mastitis. Veterinary Clinics of North America, Food Animal Practice. 19(1):157-169.

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.

Hogan, J. S., S. K.L., D. A. Todhunter, and P. S. Schoenberger. 1988. Rate of environmental mastitis in quarters infected with *Corynebacterium bovis* and *Staphylococcus* species. Journal of Dairy Science. 71:2520-2525.

Hortet, P. and H. Seegers. 1998. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Preventive Veterinary Medicine. 37(1-4):1-20.

Janowski, T., S. Zdunczyk, J. Malecki-Tepicht, W. Baranski, and A. Ras. 2002. Mammary secretion of oestrogens in the cow. Domestic Animal Endocrinology. 23(1-2):125-137.

Jayarao, B. M., B. E. Gillespie, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 1999. Epidemiology of *Streptococcus uberis* intramammary infections in a dairy herd. Journal of Veterinary Medicine. Series B. 46(7):433-442.

Jones, G. M., R. E. Pearson, G. A. Clabaugh, and C. W. Heald. 1984. Relationships between somatic cell counts and milk production. Journal of Dairy Science. 67(8):1823-1831.

Jonsson, P., S. O. Olsson, A. S. Olofson, C. Falth, O. Holmberg, and H. Funke. 1991. Bacteriological investigations of clinical mastitis in heifers in Sweden. Journal of Dairy Research. 58(2):179-185.

Kesler, E. M. 1981. Feeding mastitic milk to calves: Review. Journal of Dairy Science. 64(5):719-723.

Kingwill, R. G. 1981. The NIRD-CVL mastitis control method. Pages 24-39 *in* Mastitis control and herd management. Technical Bulletin 4. A. J. Bramley, F. H. Dodd, and T. K. Griffin, eds. College of Estate Management, Reading.

Kingwill, R. G., F. K. Neave, F. H. Dodd, T. K. Griffin, and D. R. Westgarth. 1970. The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. Veterinary Record. 87:94-100.

Klaas, I. C., C. Enevoldsen, A. K. Ersboll, and U. Tolle. 2005. Cow-related risk factors for milk leakage. Journal of Dairy Science. 88(1):128-136.

Kornalijnslijper, E., B. Beerda, I. Daemen, J. van der Werf, T. van Werven, T. Niewold, V. Rutten, and E. Noordhuizen-Stassen. 2003. The effect of milk production level on host resistance of dairy cows, as assessed by the severity of experimental Escherichia coli mastitis. Veterinary Research. 34(6):721-736.

Livestock Improvement Corporation Limited. 2005. Dairy Statistics 2004-2005, Hamilton, New Zealand.

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. 4th IDF International Mastitis Conference, Maasstricht:649-655.

Macdonald, K. and J. Roche. 2004. Condition scoring made easy. Dexcel, Hamilton, New Zealand.

Matthews, K. R., R. J. Harmon, and B. E. Langlois. 1992. Prevalence of Staphylococcus Species During the Periparturient Period in Primiparous and Multiparous Cows. Journal of Dairy Science. 75(7):1835-1839.

Matthews, K. R., R. J. Harmon, and B. A. Smith. 1990. Protective effect of *Staphylococcus chromogenes* infection against *Staphylococcus aureus* infection in the lactating bovine mammary gland. Journal of Dairy Science. 73(12):3457-3462.

McDougall, S. 1998. Efficacy of two antibiotic treatments in curing clinical and subclinical mastitis in lactating dairy cows. New Zealand Veterinary Journal. 46:226-232.

McDougall, S. 1999. Prevalence of clinical mastitis in 38 Waikato dairy herds in early lactation. New Zealand Veterinary Journal. 47:143-149.

McDougall, S., T. J. Parkinson, M. Leyland, F. M. Anniss, and S. G. Fenwick. 2004. Duration of infection and strain variation in Streptococcus uberis isolated from cows' milk. Journal of Dairy Science. 87(7):2062-2072.

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. American Journal of Epidemiology. 157(10):940-943.

Meaney, W. J. 1981. Mastitis levels in spring-calving dairy heifers. Irish Veterinary Journal. 35:205-209.

Miller, G. Y., P. C. Bartlett, S. E. Lance, J. Anderson, and L. E. Heider. 1993. Costs of Clinical Mastitis and Mastitis Prevention in Dairy Herds. Journal of the American Veterinary Medical Association. 202(8):1230-1236.

Morin, D. E. and P. D. Constable. 1998. Characteristics of dairy cows during episodes of bacteriologically negative clinical mastitis or mastitis caused by *Corynebacterium* spp. Journal of the American Veterinary Medical Association. 213:855-861.

Munch-Petersen, E. 1970. Mastitis in bovine primiparae. Veterinary Record. 87:568-574.

Myllys, V. 1995. Staphylococci in heifer mastitis before and after parturition. Journal of Dairy Research. 62(1):51-60.

Myllys, V. and H. Rautala. 1995. Characterization of clinical mastitis in primiparous heifers. Journal of Dairy Science. 78(3):538-545.

Neave, F. K., F. H. Dodd, and R. G. Kingwill. 1966. A method of controlling udder disease. The Veterinary Record. 78(15):521-523.

Newcombe, R. G. 1998. Improved confidence intervals for the difference between binomial proportions based on paired data. Statistics in Medicine. 17:2635-2650.

Nickerson, S. C., W. E. Owens, and R. L. Boddie. 1995. Mastitis in dairy heifers: Initial studies on prevalence and control. Journal of Dairy Science. 78(7):1607-1618.

Oetzel, G. R. 2004. Monitoring and Testing Dairy Herds for Metabolic Disease. Veterinary Clinics of North America: Food Animal Practice. 20(3):651-674.

Oliver, S. P., B. E. Gillespie, S. J. Ivey, M. J. Lewis, D. L. Johnson, K. C. Lamar, H. Moorehead, H. H. Dowlen, S. T. Chester, and J. W. Hallberg. 2004. Influence of prepartum pirlimycin hydrochloride or penicillin-novobiocin therapy on mastitis in heifers during early lactation. Journal of Dairy Science. 87(6):1727-1731.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, and H. H. Dowlen. 1992. Influence of Prepartum Antibiotic Therapy on Intramammary Infections in Primigravid Heifers During Early Lactation. Journal of Dairy Science. 75(2):406-414.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Jaenicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: milk production, milk quality and economic benefit. Journal of Dairy Science. 86(4):1187-1193.

Oliver, S. P. and B. A. Mitchell. 1983. Intramammary infections in primigravid heifers near parturition. Journal of Dairy Science. 66(5):1180-1183.

Oliver, S. P. and L. M. Sordillo. 1988. Udder health in the periparturient period. Journal of Dairy Science. 71(9):2584-2606.

Oltenacu, P. A. and I. Ekesbo. 1994. Epidemiological study of clinical mastitis in dairy cattle. Veterinary Research. 25(2-3):208-212.

Owens, W. E., S. C. Nickerson, R. L. Boddie, G. M. Tomita, and C. H. Ray. 2001. Prevalence of mastitis in dairy heifers and effectiveness of antibiotic therapy. Journal of Dairy Science. 84(4):814-817.

Owens, W. E., S. C. Nickerson, P. J. Washburn, and C. H. Ray. 1991. Efficacy of a cephapirin dry cow product for treatment of experimentally induced Staphylococcus aureus mastitis in heifers. Journal of Dairy Science. 74(10):3376-3382.

Owens, W. E., S. C. Nickerson, P. J. Washburn, and C. H. Ray. 1994. Prepartum Antibiotic-Therapy with a Cephapirin Dry-Cow Product against Naturally-Occurring Intramammary Infections in Heifers. Journal of Veterinary Medicine Series B. 41(2):90-100.

Pankey, J. W., P. A. Drechsler, and E. E. Wildman. 1991. Mastitis Prevalence in Primigravid Heifers at Parturition. Journal of Dairy Science. 74(5):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. New Zealand Veterinary Journal. 44(2):41-44.

Pinheiro, J., D. Bates, S. DebRoy, and S. Deepayan. 2006. nlme: Linear and nonlinear mixed effects models R package version 3.1-68.1.

R Development Core Team. 2005. R: A language and environment for statistical computing. 2.2.0 ed. R Foundation for Statistical Computing, Vienna, Austria.

Radostits, O. M., D. C. Blood, and C. C. Gay. 1984. Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 8th ed. Bailliere Tindall, London.

Rajala-Schultz, P. J., Y. T. Grohn, C. E. McCulloch, and C. L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. Journal of Dairy Science. 82(6):1213-1220.

Schepers, J. A. and A. A. Dijkhuizen. 1991. The economics of mastitis and mastitis control in dairy cattle: a critical analysis of estimates published since 1970. Preventive Veterinary Medicine. 10(3):213-224.

Schreiner, D. A. and P. L. Ruegg. 2002. Effects of tail docking on milk quality and cow cleanliness. Journal of Dairy Science. 85(10):2503-2511.

Schreiner, D. A. and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. Journal of Dairy Science. 86(11):3460-3465.

Schukken, Y. H., F. J. Grommers, D. Vandegeer, H. N. Erb, and A. Brand. 1991. Risk-Factors for Clinical Mastitis in Herds with a Low Bulk Milk Somatic-Cell Count .2. Risk-Factors for Escherichia-Coli and Staphylococcus-Aureus. Journal of Dairy Science. 74(3):826-832.

Seegers, H., C. Fourichon, and F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research. 34(5):475-491.

Shearer, J. K. and R. J. Harmon. 1993. Mastitis in heifers. Veterinary Clinics of North America - Food Animal Practice. 9(3):583-595.

Slettbakk, T., A. Jorstad, T. B. Farver, and D. W. Hird. 1990. Impact of Milking Characteristics and Teat Morphology on Somatic-Cell Counts in 1st-Lactation Norwegian Cattle. Preventive Veterinary Medicine. 8(4):253-267.

Slettbakk, T., A. Jorstad, T. B. Farver, and J. C. Holmes. 1995. Impact of milking characteristics and morphology of udder and teats on clinical mastitis in first- and second-lactation Norwegian cattle. Preventive Veterinary Medicine. 24(4):235-244.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985a. Environmental mastitis: cause, prevalence, prevention. Journal of Dairy Science. 68(6):1531-1553.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985b. Environmental Pathogens and Intramammary Infection During the Dry Period. Journal of Dairy Science. 68(2):402-417.

Sordillo, L. M. and K. L. Streicher. 2002. Mammary gland immunity and mastitis susceptibility. Journal of Mammary Gland Biology & Neoplasia. 7(2):135-146.

Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, and Y. H. Schukken. 2000. Hyperketonemia and the impairment of udder defense: a review. Veterinary Research. 31:397-412.

Timms, L. L. and L. H. Schultz. 1987. Dynamics and significance of coagulase-negative staphylococcal intramammary infections. Journal of Dairy Science. 70(12):2648-2657.

Trinidad, P., S. C. Nickerson, and T. K. Alley. 1990a. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. Journal of Dairy Science. 73(1):107-114.

Trinidad, P., S. C. Nickerson, T. K. Alley, and R. W. Adkinson. 1990b. Efficacy of Intramammary Treatment in Unbred and Primigravid Dairy Heifers. Journal of the American Veterinary Medical Association. 197(4):465-470.

Valde, J. P., L. G. Lawson, A. Lindberg, J. F. Agger, H. Saloniemi, and O. Osteras. 2004. Cumulative risk of bovine mastitis treatments in Denmark, Finland, Norway and Sweden. Acta Veterinaria Scandinavica. 45(3-4):201-210.

Venables, W. N. and B. D. Ripley. 2002. Modern Applied Statistics with S. Fourth ed. Springer, New York.

Waage, S., T. Mork, A. Roros, D. Aasland, A. Hunshamar, and S. A. Odegaard. 1999a. Bacteria associated with clinical mastitis in dairy heifers. Journal of Dairy Science. 82(4):712-719.

Waage, S., T. Mork, A. Roros, D. Aasland, A. Hunshamar, and S. A. Odegaard. 1999b. Bacteria associated with clinical mastitis in dairy heifers. Journal of Dairy Science. 82:712-719.

Waage, S., S. A. Odegaard, A. Lund, S. Brattgjerd, and T. Rothe. 2001. Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. Journal of Dairy Science. 84(2):392-399.

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. Journal of Dairy Science. 83(1):70-76.

Waage, S., S. Sviland, and S. A. Odegaard. 1998. Identification of risk factors for clinical mastitis in dairy heifers. Journal of Dairy Science. 81(5):1275-1284.

Wells, S. J., S. L. Ott, and A. H. Seitzinger. 1998. Key health issues for dairy cattle-new and old. Journal of Dairy Science. 81(11):3029-3035.

White, D. G., R. J. Harmon, J. E. S. Matos, and B. E. Langlois. 1989. Isolation and Identification of Coagulase-Negative Staphylococcus Species from Bovine Body Sites and Streak Canals of Nulliparous Heifers. Journal of Dairy Science. 72(7):1886-1892.

Woolford, M., J. H. Williamson, P. J. A. Copeman, A. R. Napper, D. S. M. Phillips, and E. Uljee. 1983. How much does mastitis affect milk production? New Zealand Journal of Agriculture. 147(1):27-34.

Woolford, M. W., J. H. Williamson, P. J. A. Copeman, A. R. Napper, D. S. M. Phillips, and E. F. Uljee. 1984. The effect of mastitis on production in the following lactation. Ruakura Farmers Conference:29-33.

Xu, Z. and L. Burton. 2003. Reproductive performance of dairy cows in New Zealand. Livestock Improvement Corporation.

Zecconi, A., R. Piccinini, and L. K. Fox. 2003. Epidemiologic study of intramammary infections with Staphylococcus aureus during a control program in nine commercial dairy herds. Journal of the American Veterinary Medical Association. 223:684-688.