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RISK FACTORS AND PREVENTION STRATEGIES FOR MASTITIS IN NEW ZEALAND DAIRY HEIFERS

This thesis is completed as a partial requirement for the Masters of Veterinary Studies (Epidemiology) from Massey University, Palmerston North, New Zealand

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Abstract

Aims

The aims of this thesis were to investigate herd level risk factors for heifer clinical mastitis and to test the efficacy of a pre-calving intervention on prevalence of post-calving IMI, incidence of clinical mastitis and somatic cell count (SCC) in heifers.

Materials and methods

A prospective survey (chapter 3) was used to collect data concerning farmers' management practices for rearing heifers and mastitis management. A proportion of herd-owners (n=250) subsequently provided data on the clinical mastitis cases in their herd occurring in the first 120 days of the subsequent lactation.

A pilot quarter level intervention study (n=1000 quarters; chapter 4) investigated the effect of pre-calving infusion of a teat sealant. Glands were randomly assigned to one of 4 treatment groups (no treatment; mammary gland secretion collection; infusion of a teat sealant; or sample collection with infusion of teat sealant) to identify the risk of each of these treatments on post-calving IMI and clinical mastitis.

A heifer level intervention study (n=1000 heifers; chapter 5, 6) investigated the pre-calving use of teat sealant infused into all four quarters and/or treatment with the injectable antibiotic tylosin. Analysis was undertaken at quarter level (chapter 5) and heifer level (including SCC data; chapter 6).

Results

The survey identified that the cumulative incidence of heifers with clinical mastitis was higher in herds with a higher per cow milk production, with more cows milked per person, in herds with a higher stocking rate, and in herds with a higher cumulative incidence of clinical mastitis in their multiparous cows. The cumulative incidence of heifers in a herd with clinical mastitis was lower in herds that managed the lactating cows in multiple groups.

The pilot study found that the presence of an IMI pre-calving increased the risk of an IMI post-calving and the incidence of clinical mastitis, relative to no IMI pre-calving. Infusion of the teat sealant reduced the risk of post-calving IMI due to *Streptococcus uberis* and the incidence of clinical mastitis. Sampling the glands pre-calving had no effect on post-calving IMI or on incidence of clinical mastitis.

The large-scale intervention study found that neither infusion of a teat sealant nor treatment with the injectable antibiotic increased the risk of cure of pre-calving IMI. Infusion of the teat sealant reduced the risk of quarter level new IMI. At both quarter and heifer level teat sealant reduced the risk of the prevalence of post-calving IMI, incidence of clinical mastitis. At heifer level the SCC was decreased throughout lactation following the use of a teat sealant. Tylosin had no effect on prevalence of IMI, incidence neither of clinical mastitis nor on SCC.

Conclusions

It was concluded that the risk of heifer clinical mastitis was associated with a number of herd level management factors and that further studies are required to elucidate the mechanisms behind these associations. Hence, it may be possible to reduce the incidence of clinical mastitis in heifers by modification of herd level management practices. Intervention with an intramammary teat sealant pre-calving decreased the incidence of new infections over this high-risk peripartum period, and may provide a useful tool for reducing the risk of subclinical and clinical mastitis in heifers.

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"We also rejoice in our sufferings, because we know that suffering produces perseverance; perseverance, character; and character, hope." Romans 5:3

So glad you got to see it finished MUM!

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Chapter 1 Literature review on heifer mastitis in dairy heifers

Introduction

Mastitis is an ongoing challenge and expense for dairy farmers and the dairy processing industry. The costs of mastitis are associated with production loss, treatment, loss of genetic potential due to premature culling, animal welfare concerns and public health affects due to poor milk composition and presence of pathogenic bacteria in milk, and increased risk of inhibitory substances.

Despite nearly a century of research into bovine mastitis, and the advancements in scientific technology and biological understanding in that time, mastitis is still the most common and expensive of all the endemic diseases affecting the dairy cow and therefore the industry (Kossaibati 2000; Seegers et al 2003).

Mastitis is inflammation of the mammary gland. It may be defined as clinical mastitis, that is where there are gross/visible physical changes in the gland and/or milk, or as subclinical mastitis, which results in no visible changes, and can only be diagnosed by the use of analytical tests (Radostits et al 1994). Mastitis can be caused by trauma to the gland or by infection with bacteria, viruses or fungi. Bacterial infection is the most common cause of mastitis. Bacterial mastitis pathogens have historically been grouped as either `contagious' or `environmental' (Blowey and Edmondson 1995). The 'contagious' pathogens are those bacteria that are adapted to survive within the mammary gland of the cow for an extended period and are typically transferred from cow to cow at, or around, the time of milking via the milkers hands or via the milking machines (Radostits et al 1994). The 'environmental' pathogens are considered as opportunistic invaders of the mammary gland and are not adapted to survival within the host; they enter the gland through the teat end also and multiply in the teat canal, which generates an immune response from the host. The primary contagious pathogens include *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus agalactiae* and the primary environmental pathogens include *Escherichia coli* and *Streptococcus uberis*. However, there is now an increasing body of evidence to suggest that this classification may not be as definite as previously thought (Bradley 2002). Bacterial mastitis pathogens may also be defined as 'major' (i.e. causing significant damage to the gland) and 'minor' (i.e. causing minimal damage to the gland, a small rise in SCC and rarely developing to clinical mastitis). The most commonly isolated major pathogens include *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus agalactiae*, *Escherichia coli* and *Streptococcus uberis*; and the most commonly recognised minor pathogens are *Corynebacterium* spp. and coagulase-negative staphylococcus.

The most common way these bacterial pathogens gain entry into the mammary gland is through the teat orifice. Therefore, the first lines of defense for the mammary gland against infections are the teat end and teat canal. If the teat end is affected by warts, sores, or teat end damage from poor milking procedure, or the teat canal is depleted of keratin lining (i.e. the teat plug formed is inadequate) then the risk of intramammary infection (IMI) increases (Capuco et al 1992; Dingwell et al 2004). Not only is the keratin teat plug a physical barrier against bacteria it also contains esterified and non-esterified fatty acids, which have bacteriostatic properties (Treece et al 1966). Cationic proteins present in the teat canal also bind to the wall of the bacteria affecting their osmolarity and leading to cell lysis (Treece et al 1966). In multiparous

cows, a teat plug occludes the teat canal and forms in the majority of cows over the dry period (Williamson et al 1995). However, whether keratin teat plugs form in the teat canal of heifer's quarters is unknown.

The immune response to bacterial infection of the mammary gland involves both a cellular and a humoral response. A number of different cell types are routinely found in milk including neutrophils, lymphocytes, macrophages and a smaller number of epithelial cells and bacterial cells. When the gland is invaded with a pathogen, large numbers of these immune cells migrate to the gland and result in a dramatic rise in the somatic cell count (SCC) from $<10^5$ cells/ml to $>10^6$ cells/ml within a few hours (Sordillo and Streicher 2002). The duration and intensity (number of cells) of the inflammatory response can have a major impact on the quantity and quality of the milk (Sordillo et al 1997) and on duration and severity of infection.

The 'soluble' or humoral responses include both innate and specific or induced components. Immunoglobulins are the soluble effectors of the humoral immune response, produced by the antigen-activated B-lymphocytes. The four classes of antibodies known to function in the immune response against bacteria causing mastitis are IgG^1, IgG^2, IgA and IgM, which are all produced locally within the mammary gland (Guidry and Miller 1896). Non-specific bacteriostatic factors within the gland include lactoferrin, lysozyme and lactoperoxidase, which work independent of antibodies and the cellular defenses.

The function of the immune system and how the immune response at quarter and cow level affects IMI are important factors in understanding some of the risk factors addressed in this literature review. However, little is know about how the immune system in heifers, which may be more naïve, functions relative to the cow and how this may affect the risk of IMI or clinical mastitis.

Review

Literature published from 1966 to 2006, in the English language, found by searching multiple electronic databases under the search 'heifer mastitis' and other specific areas relating to the topic, were considered for this review. Where possible the original scientific article was identified with reference to a specific piece of research. Peer-reviewed journal articles were given more credibility, however recent non-peer review research that was original was also included in a few cases. Recent research was emphasised because advances have been made in the understanding of this disease and in the area of prevention of mastitis, which means the more recent studies will use more similar management practices relevant to the current situation. This review has attempted to provide background information for the reader on the key objectives of this thesis, which therefore describes the current knowledge in the areas of epidemiology, prevention, and economics of subclinical and clinical mastitis in dairy heifers. For this thesis, the term heifer refers to animals approximately 18 to 36 months old, who are pregnant for the first time or in their first lactation.

Epidemiology of heifer mastitis

New Zealand studies have found the cumulative incidence of clinical mastitis over the first 5 days post-calving to be 8.1% of heifers (Pankey et al 1996) and over the first 6 weeks of lactation to be 9.9% in cows (McDougall 1998). Comparatively lactational incidences from Northern Hemisphere studies showed a wider range with 3% to 34%

incidence of clinical mastitis across all age groups (Myllys and Rautala 1995; Edinger et al 2000).

The incidence rate of clinical mastitis, especially around the time of calving, has been shown to be higher in heifers compared to cows in a number of studies (Hogan et al 1989; Barkema et al 1998a; McDougall 1999; Figure 1). Reasons for this are unclear. A survey of bovine mastitis treatments in Norway, Finland and Sweden (Valde et al 2004) found higher cumulative incidence of mastitis in heifers compared specifically to second and third parity animals in the first 2-3 weeks of lactation, but there was variability between countries. Heifer mastitis may be a problem within a herd independent of the cow mastitis incidence as heifers are yet to experience the milking procedure, are still growing therefore have higher nutritional demands and are often managed differently to the other cows in the herd. Therefore, there may be a different set of risk factors for heifer mastitis and therefore different preventive measures required.

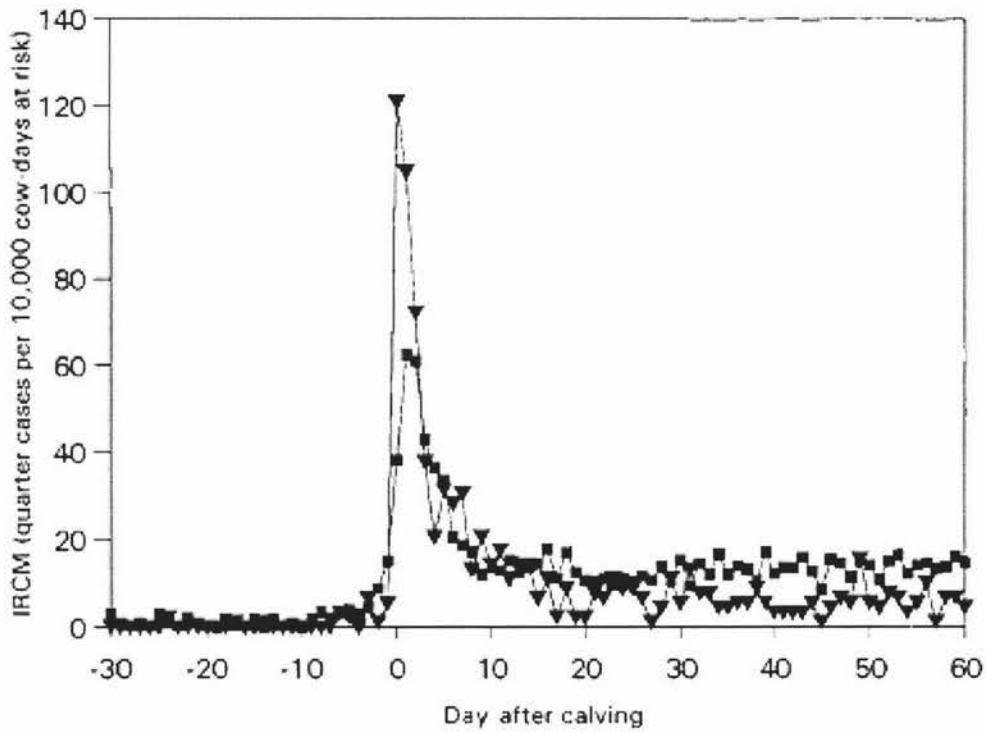

Figure 1 From (Barkema et al 1998c). Distribution of the incidence rate of clinical mastitis (IRCM) relative to the day of calving for heifers ▲ and older cows ■.

Various studies have shown that the SCC of heifers is lower than their older herd-mates throughout lactation (Schepers et al 1997). If clinical mastitis is prevented in heifers around calving then subclinical mastitis in heifers is usually not a major problem and unlikely to be contributing to a high BTSCC for the remainder of the season. The types of bacteria isolated from glands with subclinical mastitis do reflect to some extent the types of bacterial pathogens causing clinical mastitis in a herd. However the relative prevalence of major and minor pathogens is often different for subclinical and clinical mastitis cases.

Bacteriology of mastitis in heifers

Recent nationwide epidemiological studies have identified a low prevalence of *Streptococcus uberis* isolated from subclinical mastitis cases in Finland (0.65%) (Pitkala et al 2004) and Norway (0.4%; Østera et al 2006). *Streptococcus uberis* is a significant cause of subclinical and clinical mastitis in New Zealand (McDougall 1998).

The bacteria that were most frequently isolated from clinical mastitis cases in heifers in Norway were *Staphylococcus aureus* (44.3%), *Streptococcus dysgalactiae* (18.2%), *Staphylococcus aureus* together with *Streptococcus dysgalactiae* (1.2%), coagulase-negative staphylococci (12.8%), *Arcanobacterium pyogenes* (3.5%), and only 1.8% *Streptococcus uberis*. Seasonal variations were observed in the distribution of organisms with the proportion of *Staphylococcus aureus* and *Arcanobacterium pyogenes* being the highest, and the proportion of coagulase-negative staphylococcus being the lowest, in late autumn and early winter (Østera et al 2006). Waage et al, (1999) found the proportion of *Escherichia coli* IMIs to be the highest in the summer months and others have also identified regional and seasonal variations in prevalence and relative frequency of the specific IMI pathogens in heifers (Fox et al 1995). This may be related to regional variation in managerial approaches, genetics of the animals, climatic conditions and hence bacterial survival (Fox et al 1995). In North American studies, specific regional factors such as presence of the horn fly, which has been shown to transmit *Staphylococcus aureus*, may account for some regional variation (Fox et al 1995; Owens et al 2002). The prevalence of pathogens causing IMI and clinical mastitis in New Zealand dairy heifers had not been described at the commencement of this thesis. The major differences between New Zealand and other production systems are that in New Zealand most animals calve in late winter/early spring, that the majority of the cows' diet is pasture for the entire lactation, that the cattle are not housed and that the production levels are generally lower than in the more intensive production systems.

Risk factors for mastitis in heifers

Risk factors for heifer mastitis may differ from those for cows because heifers are still growing, have not previously been milked and are undergoing adaptation to the milking process. Under New Zealand management systems heifers are often raised remote from the lactating herd, thus potentially exposing them to different risk factors to those of cows. Risk factors for mastitis may be present at the quarter, heifer, herd and regional level making data analysis and interpretation of the results difficult. Additionally intervention strategies need to be developed that can be implemented at all these levels.

Herd level risk factors for heifer mastitis

There are only a few studies that have investigated herd level risk factors for mastitis in heifers, possibly because of the large numbers of herds that are required to get significant effects and hence the cost of these studies becomes prohibitive.

Known herd level risk factors for clinical mastitis in multiparous cows include environment, milking machines, teat antisepsis, human factors and culling decisions (Schukken et al 1990; Elbers et al 1998; Barkema et al 1999). In intensive systems, poor hygiene in dry period housing or faecal contamination of bedding around calving

are significant risk factors for environmental mastitis independent of parity (Smith et al 1985; Todhunter et al 1995). There are limited studies of herd level risk factors for clinical mastitis specifically in pasture-based production systems (McDougall 2003). McDougall et al, (2003) identified dry period risk factors that were associated with BTSCC and the incidence of clinical mastitis in New Zealand herds. Smaller herd size was associated with lower BTSCC, which is consistent with other studies (Barkema et al 1998b). Some of these known herd level factors for cows such as the environment are likely to be risk factors for mastitis independent of parity. However other risk factors, such as the milking machine, may change their effect size on mastitis with parity.

Differences in environmental exposures and in the predominant pathogens causing IMI i.e. *Streptococcus uberis* under NZ systems (Pankey et al 1996; McDougall 1999), and coliforms under housed systems (Barkema et al 1998a), are likely to lead to differences in the main risk factors for mastitis in seasonally calving, predominantly pasture-fed dairy production systems in New Zealand compared to year-round calving, total mixed ration fed, housed systems commonly used in Europe and North America.

Increased heifer clinical mastitis occurs with increased incidence of herd clinical mastitis (Myllys and Rautala 1995; Waage et al 1998), lower BTSCC (Myllys and Rautala 1995), and an increase in the mean milk yield (Myllys and Rautala 1995; Waage et al 1998). Barkema et al, (1998c) found that BTSCC was not correlated with incidence of clinical mastitis in a herd. For heifers, the risk of clinical mastitis increased 1.7 times in herds producing >7000L compared to herds producing <6000L (Oltenacu and Ekesbo 1994). This is consistent with the findings in other age groups (Schukken et al 1990; Grohn et al 1995). The risk of clinical mastitis also varied across regions, increased with increasing herd size, was higher with calving in the late summer and early spring and varied with composition of the diet with heifers kept on pasture in summer at decreased risk of clinical mastitis (Myllys and Rautala 1995; Waage et al 1998).

Heifer level risk factors for mastitis

At individual heifer level, known risk factors for clinical mastitis include cross suckling between heifers (Keil et al 2000), blood in the milk (Waage et al 2001), udder and teat oedema (Slettbakk et al 1995; Waage et al 2001), milk leakage at the time of calving (Myllys and Rautala 1995; Waage et al 1998; Waage et al 2001) and mechanical spread of mastitis pathogens by flies (Owens et al 2002). Breed has been identified as a risk factor for mastitis, with Friesians being at higher risk than Ayrshires (Myllys and Rautala 1995) and Jersey and crossbreds (Compton et al., unpublished). Oltenacu and Ekesbo, (1994) identified month of calving, age at calving and other diseases as risk factors for mastitis in heifers. Once identified, these risk factors for heifer mastitis may be used to recommend changes in management systems in order to decrease the prevalence of IMI and incidence of clinical mastitis and offer avenues for further research.

However, for some of the known risk factors for heifer mastitis, such as udder and teat oedema, little is known about the predisposing factor(s). There are inconsistent associations between dietary concentrate, protein intake and udder oedema, and some suggest that dietary salt intake may be more important than the proportion of concentrate of the diet (Lema et al 1992).

It has been hypothesised that feeding calves milk from cows with clinical mastitis may result in bacterial transmission either mechanically due to cross-suckling (Kesler 1981) or due to haematogenous spread to the mammary gland following absorption of ingested pathogens. The literature is not conclusive and further work needs to be done to identify if and how mastitic milk is a risk to heifers (Yagi et al 2004).

Cows with poor udder hygiene have higher SCC (Schreiner and Ruegg 2003) and poorer milk quality (Pankey et al 1987). Heifers with poor udder hygiene at calving have increased risk of post-calving IMI (Compton et al., unpublished). Historically farmers have argued that docking the tail improves udder hygiene, however, a number of studies have shown that the cleanliness of the udder is not improved with tail removal (Eicher et al 2001; Tucker et al 2001; Schreiner and Ruegg 2002). Furthermore these studies showed no difference in SCC and the prevalence of IMI in docked compared to undocked animals (Tucker et al 2001; Schreiner and Ruegg 2002).

Higher potential milk yield of an individual heifer is associated with increased risk clinical mastitis (Chassagne et al 1998; Waage et al 1998), independent of herd production levels. However, there is also a reduction in milk production associated with clinical and subclinical mastitis. Thus the relationship between milk production in the current lactation and IMI or clinical mastitis is difficult to quantify. Grohn et al., (2004) found that cows with clinical mastitis suffered a sharp drop in production, beginning before there were any signs of mastitis and the lower production level often continued for some time, with some affected cows never regaining their pre-mastitic milk yields. The loss in production was found to be pathogen specific with *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. causing the greatest declines in milk yield for the heifers. The preceding lactation's production has a clearer association with the risk of clinical mastitis. Heifers and cows with mastitis have higher production potential but their actual milk production is lower (Myllys and Rautala 1995; Grohn et al 2004).

A number of studies have demonstrated that at cow level, lower SCC cows have a higher risk of clinical mastitis (Schukken et al 1999; Suriyasathaporn et al 2000; Green et al 2004), possibly due to the lower numbers of leukocytes. Glands in which there are a higher number of leukocytes, measured as a higher SCC, may be able to remove bacteria more quickly or initiate a more effective inflammatory response than glands with low numbers of leukocytes (Suriyasathaporn et al 2000). In an *Escherichia coli* induced mastitis challenge study, milk SCC before challenge with the bacteria was correlated with stimulated CD18 expression (indicating the immune response was triggered), and both SCC and CD18 expression were inversely correlated with bacterial growth rate (Shuster et al 1996). This may suggest that a very low SCC is associated with lower immune efficacy in the mammary gland and hence an increased risk of IMI. This may be a pathogen specific effect, as cows diagnosed with clinical mastitis that isolated *Escherichia coli* were more likely to have a lower mean SCC pre-IMI, whereas cows diagnosed with clinical mastitis that isolated *Staphylococcus aureus* were more likely to have a higher mean SCC (Green et al 2004). It is possible that false negative diagnosis can occur with both *Escherichia coli,* due to its often rapid elimination from the gland and with *Staphylococcus aureus*, due to its intermittent shedding in the gland, which may affect the interpretation of the these apparent relationships between SCC and IMI status. At cow level, the relationships between individual cow SCC, risk of IMI, clinical mastitis and milk

production are complex and multifactorial, with cause and effect being difficult to partition.

Effect of existing intramammary infection on risk of new intramammary infection

In heifers, presence of an IMI pre-calving is associated with an increased risk of post-calving IMI (Aarestrup and Jensen 1997). There is some degree of persistence of pre-calving IMI in both heifers, which has been demonstrated for *Streptococcus dysgalactiae* (Aarestrup and Jensen 1997), and in cows with dry period IMIs with various bacterial spp. persisting until early/mid lactation (Green et al 2002). However, there is some evidence that presence of an IMI pre-calving may increase the risk of a new IMI. Intramammary infection in heifers post-calving may have existed in the gland for a long (i.e. months) or a short (i.e. days) period of time.

The dry period is a major risk period for new IMIs, particularly just after the last milking and approaching calving in multiparous cows (Eberhart 1986; Oliver and Sordillo 1988). This is because the udder is most resistant to IMI when it is completely involuted, partly due to the presence of a natural keratin plug in the teat canal acting as a physical barrier (Oliver and Sordillo 1988; Capuco et al 1992; Williamson et al 1995). The peripartum period has also been identified as a risk period for new IMI in heifers (Aarestrup and Jensen 1997). Therefore, some of the risk factors for IMI of multiparous cows during this period may be applicable to heifers.

Risk factors for new IMI during the dry period may operate at the quarter, the cow, and at herd level. At the level of the individual quarter, bacterial populations present at the teat end, the integrity of the teat end, and timely formation of the keratin plug are very important (Dingwell et al 2004). However, 3–5% of teat canals never close during the dry period (i.e. no teat plug), and 97% of clinical mastitis cases that occurred during the dry period occurred in quarters that did not have a keratin plug (Williamson et al 1995). Dingwell et al, (2004) found that environmental *streptococci* and coliforms were the most common cause of new IMI during the dry period (34% and 30% of all new IMI, respectively). DNA fingerprinting of *Enterobacterial* spp. indicated that these organisms persist from the dry period into lactation and can cause clinical mastitis in the subsequent lactation (Bradley and Green 2000). Dingwell et al, (2004) found that the risk of dry period IMI increased in quarters that had a cracked teat end, quarters that had not formed a keratin teat plug early in the dry period and quarters from cows with higher milk production on the day prior to drying-off. Quarters within cows with higher milk production were less likely and slower to form teat plugs.

One of the potential risk factors for IMI and clinical mastitis is previous and/or existing IMI. However, studies have provided conflicting evidence. Some studies suggest that glands previously infected with minor pathogens, in particular coagulase-negative staphylococcus, are more resistant to subsequent infection than uninfected glands (Edwards and Jones 1966; Rainard and Poutrel 1988; Matthews et al 1991). Glands infected with coagulase-negative staphylococcus showed a reduced rate of IMI following experimental challenge with *Staphylococcus aureus* compared to bacteriologically negative glands (Pankey et al 1985). This apparent protective action of minor pathogens has recently been supported by an *in vitro* study, which showed that *Staphylococcus chromogenes* taken from the teat apex of heifers consistently inhibited all the growth of *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* strains (De Vliegher et al 2004). However, in a matched case

control study, previous IMI with coagulase-negative staphylococcus was found to have no protective effect on subsequent infection with a major pathogen (Lam et al 1997).

A similar protective effect has also been demonstrated for *Corynebacterium* spp., with glands infected with *Corynebacterium bovis* less likely to become subsequently infected with major pathogens (Black et al 1972; Rainard and Poutrel 1988; Lam et al 1997). Rainard and Poutrel, (1988) found that the protective effect of coagulase-negative staphylococcus was greater than with *Corynebacterium* spp. This protective action of *Corynebacterium* spp. is not consistent as others have demonstrated that glands with *Corynebacterium bovis* are significantly more likely to become infected with environmental *Streptococcus* spp. (Hogan et al 1988) and *Streptococcus agalactiae* (Pankey et al 1985).

Green et al, (2002) attempted to investigate the conflicting evidence supporting the protective effects of *Corynebacterium* spp. and found that glands from which *Corynebacterium* spp. were isolated at drying off were at an increased risk of clinical mastitis in the subsequent lactation, whereas the presence of *Corynebacterium* spp. in the late dry and post-calving samples were associated with a reduction in the risk of IMI and clinical mastitis. One explanation is that different major pathogens, due to varying antigenic properties, may behave differently in the presence or absence of an existing IMI. A study that monitored new infection with *Staphylococcus aureus* and *Streptococcus uberis* every 3 weeks over an 18 month period during lactation found no effect of minor pathogens on subsequent infection with *Streptococcus uberis* but there was an increased risk of *Staphylococcus aureus* IMI (Zadoks et al 2001). Another possible explanation for this is that the protective effects of *Corynebacterium* spp. are not identified because the glands that are or were infected with a pathogen may be innately susceptible to repeat infection, irrespective of the protective effects of the IMI with *Corynebacterium* spp.

The reasons a gland may be more susceptible to IMI may include anatomical features such as short wide teat canal (Grindal et al 1991), low SCC (Peeler et al 2003) or poor white cell (immune) function (Hill 1981). Alternatively the effects of an IMI in the gland may be long-term. Damage to the teat canal, which may alter the normal environment of the gland may lead to an increase its susceptibility to reinfection or increase the chance of super infection (Green et al 2002). In contrast, a protective effect of minor pathogens may occur due to a small rise in gland SCC, which improves the ability to fight new IMI (Shuster et al 1996), or due to competitive exclusion of the bacteria (Bradley 2002).

Most of the research that has been conducted to try to unravel this complex interaction between previous IMIs and clinical mastitis has been carried out in multiparous cows, either specifically during the lactating or the dry period. Whether the risk of a previous IMI for a new IMI in a gland from a multiparous cow is the same, as the undeveloped heifer's gland is not known. Understanding the long-term effects of the presence of minor pathogens in the gland requires further work, especially in heifers, with the timing of infection, immunity of the animal, bacterial species of the previous or existing IMI and new IMI most likely being important to the risk of IMI and ultimately developing clinical mastitis.

Management of heifer mastitis

The prevalence of mastitis within a herd can be reduced by decreasing the risk of new infection or by decreasing the duration of infection if it does occur (Kingwell et al 1970). The 'Five-point plan' was developed to reduce the prevalence of IMI in the herd. The incidence rate of new IMI can be reduced by segregation of infected animals, hygienic udder preparation, post-milking teat disinfection and regular milking machine maintenance. Whereas culling infected cows and administering effective treatment will reduce the duration of existing infection. The 'Five-point plan' was extremely successful resulting in a reduction in prevalence of infection in cows by 65% after 3 years (Kingwell et al 1970) and gained widespread acceptance, being the basis for the American, New Zealand and Australian mastitis management plans. However the 'Five-point plan' focuses particularly on contagious pathogens and the incidence rates of new IMIs due to the environmental bacteria (e.g. *Streptococcus uberis* and coliforms) were only slightly reduced. Currently these environmental pathogens are the primary cause of mastitis in many areas, particularly when the cows are on pasture (*Streptococcus uberis*) or fed high concentrate diets on feed pads (*Escherichia coli;* Lacy-Hulbert et al 2002).

Reducing the intramammary infection pre-calving

Infusion of 'long-acting' intramammary antibiotics at the end of lactation have been used for 50 years to cure existing IMIs and to decrease the risk of new IMIs during the dry period, with the ultimate aim of reducing IMIs and clinical mastitis in the following lactation (Smith et al 1967). Williamson et al, (1995) confirmed that the administration of antibiotic dry cow therapy at the end of lactation reduced the number of new clinical IMIs (both during the dry period and in early lactation) due to *Streptococcus uberis* and reduced SCC, regardless of whether the cow or quarter were classified as infected or uninfected at drying off. This study also identified that the quarters that received dry cow therapy resulted in earlier closure of the teat canal after drying off. Therefore the gland is not only protected for a period of time with antibiotics but also physically sealed off from bacteria once the keratin teat plug is formed.

Internationally, various field studies have investigated the effects of using intramammary antibiotic treatments pre-calving in heifers for prevention of mastitis around calving. A study in Louisiana involving 73 heifers from 3 herds, with 97% prevalence of IMI pre-calving found that a penicillin and dihydrostreptomycin dry cow intramammary treatment infused >4 weeks pre-calving reduced post-calving quarter IMI prevalence, and SCC at first herd test (Trinidad et al 1990). They found the greatest impact of treatment was achieved when it was administered in the second trimester of pregnancy. Infusion of a cephapirin dry cow intramammary treatment 10-14 weeks pre-calving resulted in a > 90% cure rate of existing IMI (Owens et al 1994). They also reported that pre-calving dry cow therapy resulted in higher cure rates than treatment with a lactating cow therapy at calving for *Staphylococcus aureus* (96.0% vs. 62.5%). A second study by this same group (Owens et al 2001) with 233 heifers used 5 different dry 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 with *Staphylococcus aureus* occurred when treatment was administered in the last trimester compared to mid gestation.

Studies have also been conducted using intramammary treatments normally used for lactating cows. Infusing intramammary cloxacillin or cephapirin into the glands of

115 heifers 7 days before due calving date resulted in cure of 83% and 97% of all IMI respectively, compared to the control group, in which 29% of IMI cured (Oliver et al 1992). Penicillin-novobiocin or pirlimycin lactating cow treatments administered 14 days before due calving date significantly reduced the prevalence of IMI post-calving (Oliver et al 2004). In that study, in Jersey heifers, the cure rates were 75%, 87% and 56% for glands treated with penicillin-novobiocin or pirlimycin or those left untreated controls, respectively. A concurrent study in 55 Friesian heifers found similar efficacy with 76% of quarter's cured following treatment with penicillin-novobiocin and 59% cured following treatment with pirlimycin compared to the untreated controls (26%). The different cure rates between studies, may have been associated with the bacterial species isolated pre-calving, or may in fact be due to between herd variations.

Use of intramammary antibiotics designed for use in lactating cows or cows at their last milking (dry cow therapy), in heifers pre-calving, does not meet the Agricultural Compounds of Veterinary Medicine (ACVM) guidelines. These treatments may increase the risk of heifers producing milk with detectable antibiotic residues in early lactation. Some quarters treated with a penicillin and dihydrostreptomycin dry cow intramammary therapy, 3 months before calving, had detectable residues present in the milk 3 days after calving (Trinidad et al 1990). Oliver et al, (1992) found that 17% and 85%, of composite quarter milk samples tested positive on the day of calving for antibiotic residues in heifers treated with lactating cow cloxacillin or cephapirin approximately 7 days pre-calving, respectively. A further study (Owens et al 1994) reported no antibiotic residues detected at 5 days post-calving following cephapirin dry cow therapy 10-14 weeks earlier. Owens et al, (2001) recommended treatment 60-45 days pre-calving to give sufficient time for antibiotic residues to decrease below the inhibitory substance threshold and ensure maximum number of infections can be treated.

Significantly improved milk production and improved milk quality (lower SCC) resulting in economic benefits have been demonstrated following the use of pre-calving antibiotic therapy in heifers (Oliver et al 2003). However, these treatments may be more cost effective and applicable in countries where premiums are paid for low BTSCC, such as Australia. The efficacy of pre-calving treatment with antibiotics was found to be higher in some studies (Oliver et al 1992; Oliver et al 1997; Oliver et al 2003) than others (Trinidad et al 1990). Oliver et al, (2003) explains this could be in part due to the time pre-calving at which the heifers were treated, the difference in pathogens causing the IMIs and the time when new IMIs are occurring. Fox et al, (1995) found that incidence of IMI was highest during the last trimester, and others have found that incidence of new IMI increases closer to calving (Aarestrup and Jensen 1997); therefore treatment in the last trimester may be the most effective period to treat. It is likely that the concentrations of antibiotic will decrease below minimum inhibitory concentrations if treatment occurs at too great an interval before calving and thus increasing the risk of a new IMI before calving.

In seasonally calving dairy herds, where large groups of animals calve within a short time period and calving dates of individual animals are not known, intramammary antibiotics infused into each gland of every heifer present logistic problems. Increased risk of inhibitory substance grades in seasonal calving herds may occur because a large number of heifers are calving concurrently with the milk being sent to supply, and their actual calving date may be closer to the infusion date than anticipated. An alternative to intramammary therapy may resolve some of these issues. Repeated treatments with an intramuscular injection at fixed points in time are practically

achievable. Multiple studies have showed that intramuscular treatment with specific antibiotics is effective at treating subclinical and clinical mastitis in lactating cows (McDougall 1998; St Rose et al 2003). Williamson (2002) reported that the use of an injectable penicillin in 21 sets of identical twin heifers given once or twice within 7 days of anticipated calving did significantly reduce the percentage of infected quarters at the first milking, but that there was no significant difference in the incidence of clinical mastitis. Bryan and Friton, (2004) reported that the use of intramuscular penethamate in heifers pre-calving decreased the number of treated heifers with clinical mastitis at calving. However no large, peer reviewed studies have been reported using an intramuscular treatment for mastitis prevention in heifers pre-calving.

Reducing the risk of new intramammary infection in heifers

Decreasing teat end exposure to bacterial pathogens through application of a pre and post-milking teat disinfectant is effective at decreasing IMI during lactation (Oliver et al 1994; Oliver et al 1999). However, repeated teat disinfection pre-calving has not proven successful with the products tested. Edinger et al, (2000) reported no significant protective effect against IMI from using an iodine-based barrier teat dip starting approximately 12 days prior to expected calving in heifers. The study was in only one herd and the major pathogen isolated was *Staphylococcus aureus,* suggesting the barrier dip used may not have been effective against contagious pathogens. However the statistical power to show a significant reduction in IMI with only 149 heifers would be low. In a recent New Zealand study, using a teat sanitiser applied as a spray three times a week pre-calving has been shown to reduce *Streptococcus uberis* numbers by 40% at the teat end (Lopez-Benavides, pers comm). More research needs to be performed in this area to improve the duration of action of the products to make them less labour intensive.

External teat sealants are a non-irritant latex, acrylic or polymer-based film that are applied like a teat dip to produce a layer over the teat end that prevents entry of bacteria into the teat canal. External teat sealants avoid any potential for damage to the teat canal or accidental introduction of pathogens following intramammary infusion via the teat canal. Farnsworth et al, (1980) demonstrated that in lactating cows an external teat sealant remained intact until the milking following its application and the rate of new infection of *Staphylococcus aureus*, *Staphylococcus epidermidis* and coliforms in treated quarters was reduced by 28%, 33%, and 76%, respectively. However, a within cow field study tested the efficacy of a latex teat sealer in 32 animals and found that new infections with coliforms, *Staphylococcus spp.,* or *Streptococcus spp.* were not reduced in treated quarters (McArthur et al 1984). The power to find a reduction in new IMI with this small number of animals would be low. The application of an external teat sealer once at the end of lactation and again at 10 days pre-calving, with reapplication as required to maintain coverage at all times until calving, resulted in a significant reduction in dry period new IMI incidence in two studies (Timms 2001). This protocol resulted in a reduction of new IMI for all pathogens over the non-lactating period of 26% in heifers and 34% in cows. The dip was equivalent to infusion of dry cow antibiotic therapy for prevention of early dry period IMI for both major and minor pathogens (Timms 2001). Problems with the duration of adherence of the external teat sealers have prevented widespread uptake of this technology. The average adherence time, with the teat end covered was found to be six days, however environmental and management conditions may affect this (Corbellini et al 2002).

The use of an internal physical barrier infused into the teat canal and teat sinus at drying off was first examined in the early 1970s (Meaney 1977). The composition of this compound was bismuth subnitrate in a paraffin base (37% w/w), which was an inert viscous paste, insoluble in milk, and found to have no antimicrobial properties or food safety or residue risks. It was of high relative density but did not set or solidify in the teat, being removed at calving either by the sucking action of the calf or by manual stripping at first milking. The seal was intended to fill the fissures and folds within the teat canal and the lower teat sinus and so provide a physical barrier to the ingress of pathogens during the dry period (Woolford et al 1998). A 90% reduction in the incidence of new IMIs during the dry period occurred following infusion of teat sealant at the end of lactation where the teats were challenged with a bacterial broth (Meaney 1977). The sealant remained in the distal teat canal for at least 3-4 weeks after drying off as demonstrated by radiography (Meaney 1977). Little of the material was lost via the teat orifice during the entire dry period, although some intramammary dispersion did occur as the mammary gland underwent lactogenesis (Meaney 1977). It is likely that the teat sealant remains in place until it is physically removed from the gland usually at the start of lactation. Radiographic images identified the presence of the teat sealant in the teat cistern for up to 106 days after infusion (Woolford et al 1998), this suggests that flecks of teat sealant could be visible in the milk for up to this period of time (Huxley et al 2002).

Further work has confirmed that infusion of a bismuth teat sealant (65% w/w, 2.6 g) at the end of lactation reduces new IMI rate over the dry period in multiparous cows (Woolford et al 1998; Berry and Hillerton 2002; Huxley et al 2002). Infusion of the teat sealant following dry cow antibiotic therapy results in a 30% reduction in risk of a new IMI between dry off and 1 to 3 DIM, 33% reduction in risk of clinical mastitis event between dry off and 60 DIM, and lower linear SCC score compared to control quarters that were treated only with the dry cow therapy (Godden et al 2003). The absence of antibiotic action of the teat sealant means that it is not recommended for use in cows with existing infection. Drug registration guidelines recommends that teat sealant be used in animals with SCC <150 000 cells/ml. Use of a teat sealant alone reduces the risk of antibiotic residues in milk, which makes it an attractive alternative to dry cow therapy for farmers for use in low SCC cows.

Despite the success of studies in New Zealand and the United Kingdom successful use of teat sealant presents some practical difficulties. Infusion of quarters with an internal teat seal, especially without concurrent antibiotic, requires a fastidious hygienic technique to avoid introducing pathogens. Furthermore, quarters with existing IMI at the time of drying off would still require dry cow therapy to achieve elimination of these existing infections. The lack of sensitivity and specificity of tests that differentiate uninfected from infected quarters at the time of drying off may continue to limit the use of teat sealant. The use of teat sealant pre-calving has not been investigated in heifers.

Reducing the prevalence of udder oedema (a key risk factor for clinical mastitis in heifers) may lead to a reduction in prevalence of IMI and incidence of clinical mastitis. Pre-calving milking of 246 heifers resulted in reduced incidence of udder oedema pre-calving, reduced prevalence of IMI on the day after calving, reduced cumulative incidence of clinical mastitis by 135 days of lactation and increased milk production/day during the first 135 days of lactation (Santos et al 2004). One of the concerns with this intervention is the negative impact on the nutrition and health of the heifer, which may lead to decreased production and increased susceptibility to

disease (Mallard et al 1998). Blood levels of β-hydroxybutyrate (BOH) and non-esterified fatty acids (NEFA) pre-calving were higher in the pre-milked cows and pre-calving milking doubled the risk of subclinical ketosis. More research needs to be done to identify the risk factors of udder oedema in order to understand the true efficacy and economics of milking heifers pre-calving as one possible preventive strategy.

Improving immunity of cows to decrease intramammary infection

Improving heifer immunity both specifically via vaccination against mastitis pathogens (Sordillo et al 1997), and non-specifically via optimising health and minimising stress around calving, has the potential to reduce IMI and therefore clinical mastitis (Mallard et al 1998).

The perfect mastitis vaccine would decrease establishment of new IMI, eliminate chronic infection and decrease the severity of infection once established (Sordillo et al 1997). However most current mastitis vaccines only decrease the severity of clinical mastitis. Vaccination may result in more rapid recruitment of neutrophils to the mammary gland with the result that the production of specific antibodies occurs more rapidly. These antibodies are integral in opsonising the bacteria, which leads to promotion of phagocytosis by neutrophils, neutralization of bacterial toxins, interference with the adherence mechanisms of bacteria and inducing cell lysis. The only commercially available mastitis vaccine is against *Escherichia coli*, and is available in the Northern Hemisphere. Due to the low prevalence of *Escherichia coli* mastitis in New Zealand it has not been registered here.

Vaccination against *Streptococcus uberis* would be of significant economic benefit to the New Zealand dairy farmer and industry. Immunisation with live *Streptococcus uberis* by the sub-cutaneous route, along with administration of a soluble preparation of bacterial cell wall antigens by the intramammary route, resulted in some protection against subsequent experimental challenge (Hill et al 1994), which was confirmed by others (Finch et al 1997). They found that the protective effects were only against the vaccinated strain of *Streptococcus uberis,* which limits its use due to the large number of strains associated with clinical mastitis (Hill and Leigh 1989; McDougall et al 2004). The protective action of the live vaccine did not appear to be associated with an increase in neutrophils influx to the gland, which challenges the view that killing *Streptococcus uberis* by neutrophils was essential for the control of IMI (Leigh 2000). One possible explanation for the vaccine's protective effect was that vaccination had decreased the rate at which *Streptococcus uberis* could colonise the gland, which offered an alternative hypothesis around which research on *Streptococcus uberis* could be focused (Leigh 2000).

Ultimately an effective vaccine against *Streptococcus uberis* needs to effective against a range of *Streptococcus uberis* strains and reduce the incidence and severity of clinical mastitis. If this can be achieved then there is potential for significant economic benefits for the New Zealand dairy industry.

In the past 20 years there has been extensive research into the effects of nutrition on immune function. The role of selenium (Se) and vitamin E are probably the best characterised. Most of the research in this area has been done in intensive systems of the Northern Hemisphere (Smith et al 1984; Hogan et al 1993; Weiss et al 1997; Zanetti et al 1998; Hemingway 1999). Selenium is an integral component of the enzyme glutathione peroxidase, which functions as an antioxidant to protect cells

from oxidative damage. Deficiencies in Se lead to compromised function of neutrophils, and supplementation with Se leads to improved bactericidal action of neutrophils and decreased severity and duration of mastitis (Erskine et al 1989; Hogan et al 1993). Vitamin E plays a regulatory role in the biosynthesis of inflammatory mediators. The killing ability of blood neutrophils has been correlated with the concentration of α-tocopherol (α-T) in neutrophils (Weiss et al 1994). Vitamin E is present in highest concentrations in green foliage, therefore in pasture-based farming systems it is likely that vitamin E concentrations in the diet are adequate to meet dietary requirements. However, the presence of high concentrations of polyunsaturated fatty acids in lush green grass increases the oxidant challenge, therefore increasing the requirements of these nutrients (Wichtel et al 1996). The actions of Se and vitamin E are synergistic; the greatest positive responses have been observed when both adequate Se and vitamin E were supplemented (Smith et al 1984). More research in this area needs to occur to understand the added benefits of supplementation with both vitamin E and Se especially in animals, which appear to be on a diet with adequate intakes of both nutrients.

Other nutrients such as vitamin A, and its precursor β-carotene, copper and zinc have all been linked to immune function, with deficiencies associated directly with mammary gland infection, or more broadly with various effects on components of immune function. Maintaining high quality nutrition such that dietary requirements for protein, energy, fat, mineral and vitamins area are adequately met and minimizing stress around calving will minimise the chance of unnecessary disease in the herd.

Economics of mastitis

Milk quality should be a primary focus for any dairy industry. Mastitis imposes significant costs to the dairy producers and the milk processing industry (Wells et al 1998; Bradley 2002; Seegers et al 2003). Infection of the mammary gland changes the composition of the milk produced by increasing the cellular component (i.e. SCC) and decreasing production (Bennedsgaard et al 2003).

Quantifiable financial losses occur due to the cost of treatment and prevention, loss of cows due to death and premature culling from the herd, loss of milk for supply during and after treatment with antibiotics, and loss of milk production associated with elevated SCCs and potential decreased value of cows and herds. Intangible losses include the labour costs of treating cows, the increased risk of an inhibitory substance grade while treating cows with antibiotics and the increased risk of other diseases. The losses associated with clinical mastitis are estimated to be US$100-300 per cow per lactation (Schepers and Dijkhuizen 1991) and GB£175 per case (Kossaibati 2000). A recent review of the costs of common diseases of dairy cows to the UK industry (Bennett et al 1999) reported costs of mastitis to be approximately 3 times that of the next most costly disease (lameness). However, the variability in the methods used to estimate the costs of diseases such as mastitis, leads to a large variation in the estimated costs of this disease, which does create difficulties when recommending the benefits of specific management options. Also it is likely that some costs associated with mastitis may be industry specific such as the monetary value of cows, BTSCC or milk quality incentives from the milk company and drug and treatment costs.

Identifying and implementing preventive strategies for mastitis should be far superior to treating clinical and/or subclinical cases of mastitis from a cost, animal health, environmental, resource and human health perspective. Allore and Erb, (1998) using a

dynamic discrete event stochastic model, identified the most cost effective management strategies for different pathogen types. They found that preventive strategies (including milking hygiene, milking equipment maintenance, types of housing and bedding and nutrition) together with use of dry cow therapy to be the most cost effective combination for contagious pathogens. However, for environmental pathogens, vaccination against *Escherichia coli* needed to be included to make the combination most cost effective.

High individual cow SCCs and/or clinical mastitis in heifers results in lower production during their first (De Vliegher et al 2005), and subsequent lactations (Woolford et al 1983; 1984), increased susceptibility to clinical mastitis in the following lactation (Rupp et al 2000) and increased risk of premature removal from the herd (Erb et al 1985; Myllys and Rautala 1995; Rupp and Boichard 2000).

It is possible that the economic loss due to mastitis occurring in heifers could be greater than in multiparous animals. This is perhaps because heifers should be the highest genetic merit animals in a herd and therefore premature culling is a relatively large component of loss of resource. Potential production loss incurred as a consequence of clinical or subclinical mastitis may be greater in heifers compared to older cows, as there is the possibility of long-term effects. The future profitability of an animal is recognised as the retention pay-off (RPO). It is an economic index, which depends on parity, stage of lactation, pregnancy, milk production, and other factors (Houben et al 1994; Swinkels et al 2005a; Swinkels et al 2005b). The cost of growing a heifer to parturition is significant (~ NZ$1200) and if mastitis occurs during the first lactation the animal has not contributed financially to the herd-owner.

The annual gross cost of mastitis to the New Zealand dairy industry (3.8 million cows) has been estimated as between NZ$60 to NZ$189 million (McDougall pers com). Heifers represent 20%-25% of most herds (Anon 2001, www.lic.co.nz), which is approximately 800,000 heifers nationally. It is estimated that a gross saving of NZ$8.0 million/annum could be achieved by reducing heifer clinical mastitis incidence from 7.6% to 3.7% and by reducing the cull rate for mastitis in heifers from 2% to 1%, when assuming the cost of clinical mastitis is approximately $150/case. This does not include any longer term benefits of reduced SCC or increased production. Therefore, significant benefits may arise if cost effective preventive strategies are identified and developed specific to heifers.

Summary

Intramammary infection and clinical mastitis in dairy heifers is a common and expensive problem. Relative to the amount of information about bovine mastitis in general, little is known about risk factors for pre-calving IMI, post-calving IMI and clinical mastitis in heifers. Deficiencies in this knowledge area lead to difficulties in recommending reliable and economic preventive measures to farmers with heifer mastitis problems. Additionally much of the research in this area has been performed in intensive farming systems in the Northern Hemisphere, and it is difficult to know how applicable this research is, when many of the risk factors for mastitis are management-system related.

Current milk quality programmes focus on control of contagious pathogens in multiparous animals, possibly because of the lack of research in the area of environmental pathogens in heifers. The use of pre-calving antibiotics is an effective

preventive option for heifer mastitis, however the use of antibiotics prophylactically does have potential negative effects. Further work to investigate the risk factors of heifer mastitis in pastures based systems and the preventive strategies to control heifer mastitis is important.

Using epidemiological methodologies to identify risk factors for diseases such as mastitis is a cost effective and efficient research approach. Observational studies may be useful in identifying potential causal associations. These associations can be evaluated by further controlled studies which may also provide useful information on possible interventions and economics. Investigating these risk factors further can be used to formulate recommendations for prevention of mastitis or to direct further research.

The first study (Chapter 3) is a survey that investigated the relationships between herd level management factors and clinical mastitis in heifers. Heifer level risk factors were investigated in prospective cohort study that was undertaken by Chris Compton in conjunction with the intervention studies in Chapters 5 and 6. Chapter 4 was a pilot study investigating the use of a teat sealant as a preventive strategy for heifer mastitis. The effect of sampling the mammary gland pre-calving was investigated in this study so that further work could be done in the area of pre-calving IMI epidemiology. Chapters 5 and 6 used data from the same large-scale intervention study that investigated the use of teat sealant and tylosin pre-calving in heifers. Analysis was performed at both quarter (Chapter 5) and heifer (Chapter 6) level. The quarter level analysis allowed us to investigate the new infection and cure rates following the treatments, whereas the heifer level analysis is useful to be able to provide some crude economic analysis for these interventions by including the effect of the interventions on somatic cell count and production data and therefore allow recommendations to farmers.

At the time of printing of this thesis Chapter 4 in a slightly modified form had been accepted for publication in the Journal of Dairy Science, Chapter 3 had been submitted to the New Zealand Veterinary Journal and is in the process of review by the authors to be accepted for publication, and Chapter 5 and 6 are yet to be submitted. Chapter 5 (quarter-level analysis) will be submitted to the Journal of Dairy Science and Chapter 6 (heifer-level analysis) to the New Zealand Veterinary Journal.

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Chapter 2 Statistical methods to account for correlation within groups

Biologically it is plausible that quarters within a cow and a cow within a herd are more closely correlated than a quarter within a different cow or a cow within a different herd. Adkinson et al, (1993) found that quarters tended to be more alike with regard to the risk of clinical mastitis within a cow than expected based upon the assumption of independence between quarters. They suggest that this clustering may have an impact on experimental design and data analysis of quarter level studies. If clustering is ignored the then the effect of treatment on the outcome is more likely to be significant (type I error; see later) and therefore data analysis needs to account for this estimation error. Likewise study design needs to account for this relationship, for example quarter level designs need to be carefully planned.

The measures used to assess the degree of correlation or clustering within a group include the intraclass correlation coefficient (ICC), which is a proportion of the variance between the different levels, the variance inflation factor (VIF), calculated from the ICC and overdispersion, which is calculated from the variance and the mean of the data. The size of the effect of clustering on the variance estimate depends on both the ICC and size of the clusters (e.g. 4 quarters; Dohoo et al 2003, pg. 459-542). An ICC of 0 indicates no correlation within the group, an ICC of 1 indicates complete within-group clustering. An ICC of 0.1 is considered a low ICC and indicates that most of the variation is within the group, however with a large cluster size this can have a similar impact to a high ICC with a small cluster size. This needs to be considered when multiherd studies are being performed, as to reduce the effects of clustering a small number of animals from a large number of herds is preferable, although economically may not be achievable. An ICC of 0.5 is considered as a high ICC and indicated that there is little variation within the group relative to between the groups. It is generally accepted that an ICC <0.2 indicates that the degree of correlation within the group is low (Heuer pers com). The VIF can be calculated using the ICC and the number of subjects in the group, which can then be used to make the necessary adjustments to the standard errors of the estimates. If the standard errors were to be adjusted <20% then clustering is not considered to be adjusting the estimates of the model significantly.

The principal problem with ignoring clustering is that if subjects within the same cluster (e.g. cows within the same herd) have correlated responses, then the standard error and parameter variance estimates are too small such that the null hypotheses are too easily rejected (i.e. a type I error; (McDermott et al 1994). Ignoring the hierarchical structure of the data can also lead to an overestimate of the power for evaluation of higher level factors (such as herd), because it is the number of clusters (herds) that determines the degrees of freedom, not the number of subjects (cows) within a cluster (herd; Dohoo et al 2003). Dohoo et al, (2003, pg 459-542) goes on further to say that if the numbers of subjects within a cluster are highly variable then unadjusted analysis will give more weight to the cluster with more subjects. Some statistical methods to account for within-cluster correlation are available; however, a review of veterinary epidemiology papers revealed that many studies ignore herd clustering or use cluster adjustment methods developed for normally distributed data

when analyzing proportion or count data (McDermott and Schukken 1994; McDermott et al 1994). Within-herd correlation is likely to differ between herds, as management differs between herds (Zadoks et al 2001). When a common within-herd correlation cannot be assumed, mixed models with fixed effects for herd and additional random effects for subgroups (cows) within herds are needed (McDermott et al 1994).

Barkema et al, (1997) found that for the most common mastitis causing bacterial species, IMI in 0, 2, 3, or 4 quarters of the same cow occurred at a higher rate than would be expected based on independence of the quarters. *Corynebacterium bovis* and *Streptococcus agalactiae* had the highest ICC within cow, and other *Streptococcus* spp. had the lowest (Table 1). They also investigated the correlation of IMI within herd and *Corynebacterium bovis*, *Streptococcus agalactiae*, and *Staphylococcus aureus* had the highest ICC within herd and *Streptococcus uberis* had a very low ICC within herd. The differences between species may be explained by the epidemiology of the individual pathogen. *Streptococcus agalactiae*, and *Staphylococcus aureus* are considered as contagious pathogens transmitted from cow to cow; therefore it is likely that if one quarter is infected with a contagious pathogen there is an increased chance of the other quarters being infected. Intramammary infection with *Corynebacterium bovis* is often an indicator of poor milking hygiene, particularly poor quality teat disinfection; therefore if one quarter is susceptible to IMI then all quarters within a cow and a herd are likely to be at higher risk of infection.

	Herd	Cow within herd	Error	ICC	
				Within herd	Within cow
Natural logarithm of SCC	0.173973	1.339574	1.517270	0.06	0.47
Streptococcus dysgalactiae	0.000602	0.001561	0.015787	0.03	0.09
Streptococcus Lancefield group D	0.001583	0.002896	0.024477	0.05	0.11
Streptococcus agalactiae	0.000329	0.000852	0.002215	0.10	0.28
Streptococcus uberis	0.000073	0.001016	0.005703	0.01	0.15
Other streptococci	0.003053	0.002718	0.031101	0.08	0.08
Staphylococcus aureus	0.002229	0.004763	0.033876	0.05	0.12
Coagulase-negative staphylococci	0.008797	0.020141	0.091822	0.07	0.18
Corynebacterium bovis	0.023866	0.046445	0.138183	0.11	0.25
Bacillus spp.	0.001489	0.001394	0.010290	0.11	0.12

Table 1 From (Barkema et al 1997). Variance components and intraclass correlation (ICC) for infected quarters within cow and within herd. ICC >0.2 is considered large enough to require analysis to account for clustering of the data.

Streptococcus uberis, however, is an environmental pathogen and therefore increased risk of infection associated with increased faecal contamination may be independent of quarters, cows and herds, therefore the within cow and herd ICCs are lower than for some of the contagious pathogens. The within cow ICC is larger (0.15) than the ICC for *Staphylococcus aureus* (0.12), which contradict this explanation. However the risk factors for infection for a quarter with *Streptococcus uberis* may be similar between quarters in some cows and in some herds. Zadoks et al, (2001), found that exposure to other quarters infected with *Streptococcus uberis* within a cow was

associated with increased rate of infection with *Streptococcus uberis* and likewise exposure to other quarters infected with *Staphylococcus aureus* within a cow was associated with increased rate of *Staphylococcus aureus* infection. They state that because clustering at cow level and several cow and quarter-level risk factors associated with susceptibility were accounted for in their statistical models, this suggests that within-cow transmission does occur for both pathogens, however it was not quantified in the study.

The degree of clustering within a cow or within a herd may be dependent on the bacterial pathogens present and the types of management systems operating. Ensuring that clustering is identified and the analyses are selected to adjust for it, gives the author and readers confidence in the predictions made by the statistical model.

In the chapters in this thesis where correlation within cow and within herd was identified, herd was included as a random effect statement to account for correlation within herds, and heifer nested within herd was included as a random effect statement to account for correlation within cow. However when the correlation within cow was due to repeated measurements from the same cow over a period of time, an autoregressive repeated statement to adjust for correlation between measurements within cow was, instead of the random effect statement. The autoregressive statement accounts for measurements taken closer together (i.e. first and second milk test) being more correlated than measurements further apart (i.e. first and third milk test), which is biologically plausible with an outcome such as milk yield or SCC.

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Chapter 3 Dairy heifer management and its relationships with incidence of clinical mastitis

Abstract

AIMS:

The aims of this study were to describe the management of heifers before calving and to determine risk factors for postpartum heifer clinical mastitis at herd level under pasture based New Zealand management systems.

MATERIALS AND METHODS:

Dairy herd-owners (n=578) provided information via a prospective survey about their practices for rearing heifers and mastitis management. A proportion of herd-owners (n=250) subsequently provided data on the clinical mastitis cases in their herd including the date, cow number, quarter and age from cases occurring in the first 120 days after the planned start of calving in the subsequent lactation. The relationship between management factors and the proportion of heifers diagnosed with clinical mastitis within a herd was analysed by bivariate and multivariate analysis.

RESULTS:

The average herd cumulative incidence of mastitis was 13.6% for heifers and 9.0% for multiparous cows. The proportion of heifer mastitis was higher in herds with higher per cow milk production, in herds where more cows were milked per person, in herds with a higher stocking rate, and in herds with a higher cumulative incidence of clinical mastitis in their multiparous cows. Proportion of heifers in a herd with clinical mastitis was lower in herds that milked their lactating cows in multiple groups.

CONCLUSIONS:

It was concluded that the risk of heifer clinical mastitis was associated with a number of management factors and that further studies are required to elucidate the mechanisms behind these associations.

CLINICAL RELEVANCE:

Significant relationships were found between some management practices and the incidence of clinical mastitis. Hence it may be possible to reduce the incidence of clinical mastitis in heifers by modification of herd level management practices.

KEYWORDS:

Clinical mastitis, heifer, management practices

ABBREVIATIONS:

AHC Animal Health Centre
EDVS Eltham District Vet Services
ICSCC Individual cow somatic cell count
BTSCC Bulk tank somatic cell count
IMI Intramammary infection
PSC Planned start of calving

Introduction

High individual cow somatic cell counts (ICSCCs) and/or clinical mastitis in heifers results in lower production during their first lactation, long term production loss (Woolford et al 1983; 1984), increased susceptibility to clinical mastitis in the following lactation and increased risk of premature removal from the herd (Myllys and Rautala 1995; Rupp et al 2000; Rupp and Boichard 2000). The incidence rate of clinical mastitis, especially around the time of calving, has been shown to be higher in heifers than in cows (Hogan et al 1989; Barkema et al 1998; McDougall 1999). Reasons for this are, however, unclear.

At individual heifer level, known risk factors for clinical mastitis include cross suckling between heifers (Keil et al 2000), blood in the milk (Waage et al 2001), udder and teat oedema (Slettbakk et al 1995; Waage et al 2001), milk leakage at the time of calving (Myllys and Rautala 1995; Waage et al 1998; Waage et al 2001), and mechanical spread by flies (Owens et al 2002). Waage et al. (1998) investigated the effect of herd level risk factors on heifer clinical mastitis and found that increased incidence of clinical mastitis in the herd, lower bulk milk somatic cell counts (BTSCC) and increased mean milk yield were associated with increased risk of mastitis. The risk of clinical mastitis also varied across regions, increased with increasing herd size, was higher with calving in the late summer and early spring and varied with composition of the diet with heifers kept on pasture in summer at decreased risk of clinical mastitis.

In multiparous animals herd level risk factors for clinical mastitis include the environment, milking machine, teat disinfection, human and culling decision factors (Schukken et al 1990; Elbers et al 1998; Barkema et al 1999a). No studies have specifically examined herd level risk factors for clinical mastitis in heifers in pasture-based systems. Risk factors for heifers may differ from those for cows because heifers are still growing, have not previously been milked and are undergoing adaptation to the milking process. Under New Zealand management systems, heifers are often raised remote from the lactating herd potentially exposing them to different environmental risk factors to the cows. Additionally, there are limited studies of herd level risk factors for clinical mastitis in pasture-based production systems. Differences in environmental exposure and in the predominant environmental mastitis pathogen (*Streptococcus uberis*) under New Zealand systems (Pankey et al 1996; McDougall 1999) compared to coliforms in housed systems (Barkema et al 1998) suggests that it is likely that risk factors will be different in the seasonally calving, predominantly pasture-fed dairy production systems in New Zealand compared to the year-round calving, total mixed ration-fed, housed systems commonly used in Europe and North America. Identifying herd level risk factors specific to clinical mastitis in heifers potentially allows recommendations about management practices that can be used to decrease the incidence of heifer mastitis.

The objectives of this study were to identify the proportion of heifers and cows with clinical mastitis within a herd, to describe pre- and post-calving heifer management practices, and to examine the association between these management factors and the occurence of clinical mastitis in seasonally calving, pasture-fed dairy heifers.

Materials and Methods

A prospective mail survey (Appendix 1) was undertaken of owners of seasonally calving dairy herds using the services of two veterinary practices, one in the Waikato region of New Zealand (Animal Health Centre (AHC)) and one in the Taranaki region of New Zealand (Eltham District Veterinary Services (EDVS).

The survey had two parts; initially, herd-owners were questioned about rearing, nutritional and mastitis management preceding the commencement of lactation, and then a follow-up survey was undertaken to obtain the clinical mastitis records from the first four months of the subsequent lactation.

This study design had two purposes; it was both a survey, with the aim of identifying the proportion of heifers within a herd with clinical mastitis (descriptive), and a cohort observational study, where the exposure factors (management practices) were recorded prior to the period of occurrence of disease, with the aim of identifying relationships between management practices and the proportion of heifers with clinical mastitis.

The sampling frame included all active clients (i.e. herds that used veterinary services in the six months before the first survey) who owned dairy cattle and were serviced by the AHC or EDVS. The clients of AHC were located from Raglan (latitude –37.800 longitude 174.883) to Te Aroha (latitude –37.583 longitude 175.733 and from Tirau (latitude –37.983 longitude 175.750) to Ohinewai (latitude –37.483 Longitude 175.167). Clients of EDVS were located from Eltham (latitude -39.429 longitude 174.300) to Hawera (latitude -39.592 longitude 174.283) and from Opunake (latitude –39.456 longitude 173.858) to Kaponga (latitude –39.443 longitude 174.150).

The initial survey was mailed out in June (for AHC herds) or July (EDVC herds) 2003, to precede the 2003/2004 lactation. A letter explaining the purpose of the study and offering entry into a competition to win a prize accompanied the survey. A reminder letter and another copy of the survey were mailed out to non-responders approximately four weeks after the first survey. The survey was addressed to the person making the policy decisions on farm where this was known. For example, where a sharemilker was known to be managing the stock on the farm the survey was addressed to him/her rather than the landowner.

The initial survey asked questions related to the following:

- Farm information
    a. Farm area
    b. Type and size of milking parlour
    c. Number of people milking
    d. Number of cows and heifers to calve, and calendar date on which calving was expected to commence ("planned start of calving"; PSC) for both age groups
    e. Total milk solids (fat and protein) production for the herd in the previous (2002/2003) lactation
- Pre-calving and calving management of the cows and heifers including how many groups the herds were managed in, when management groups were formed relative to PSC, intervals from calving to calf removal and to first post-calving milking;

- Source of heifers (i.e. replacement animals reared from herd-owners stock or purchased), pre-weaning nutrition including source and type of milk or calf milk replacer, feeding of antibiotic-contaminated milk or milk from cows with mastitis; site of post-weaning management (i.e. on the same land area as lactating cows or grazed remote from the lactating cows);
- Management of the heifers around calving, including pre-calving training in the milking parlour, management of the heifers as a separate group from lactating cows before and/or after calving, removal of the tail at the level of the vulva;
- Mastitis management
    a. Teat antiseptics applied (active ingredient, whether applied pre or post milking);
    b. Policy for diagnosis and treatment of cows with subclinical mastitis
    c. Clinical mastitis diagnostic procedures undertaken in early lactation, and
    d. Use of oxytocin to aid in milk ejection in heifers.

Herd-owners were provided with a custom data-recording sheet to collect the clinical mastitis information on for the lactation. Data required included date of diagnosis, cow number, quarter and age (heifer yes/no). Calving date of the animals diagnosed with clinical mastitis was not requested. The authors believed that requesting this information may lead to non-compliance by the farmers. The other information that was requested could be directly obtained at the cow (cow-side), where as calving date information may not be readily accessible. This recording sheet was included as part of initial mail out and the herd-owners were informed that this data would be requested later in lactation (Appendix 1). The records were requested by mail in December 2003. Two follow up letters were sent to non-responders, and reminders were published in the AHC monthly newsletter. EDVS telephoned clients following the first mail out.

Data handling and statistical analysis

The responses to the initial survey were entered into a custom built database (Microsoft Access, 2000) which incorporated procedures designed to identify unusual values. Only herds commencing calving between mid June and late August were included in the analysis. The data were examined for outlying values and the mean, standard deviation, minimum and maximum determined for continuous variables and frequency was calculated for categorical variables. It was hypothesised that the design variable region (i.e. Taranaki or Waikato) may be a confounder in subsequent analysis, hence differences between the regions were examined using simple independent sample t-tests, and the variable region remained in all further modelling. The subset of herds (n=250) that were used in the clinical mastitis model was compared to the whole survey population (n=578) using simple t-tests for the continuous variables and chi-squared analysis for the categorical variables. The proportion of a herd with clinical mastitis was analysed by age (heifer vs. cow) and by calendar month of diagnosis using the difference of proportions tests (chi-squared) for each month.

Relationships between the management practices as determined from the initial survey and the proportion of heifers diagnosed with clinical mastitis over the first 120 days after the start of the seasonal calving period for each herd were examined. Where a heifer had multiple glands with clinical mastitis diagnosed on the same day, it was counted as one case. If a heifer failed treatment and was diagnosed with another case

of clinical mastitis, or another quarter was diagnosed with clinical mastitis on a later date then it was counted as a second case. As the risk period of exposure was not known for each heifer (calving date data was not collected) and the number of repeat cases was low (12.6%), the data was treated as a binomial outcome (clinical mastitis yes/no).

The outcome variable was therefore the proportion of heifers present on the farm at the commencement of the calving period that were recorded with clinical mastitis during the first 120 days following the planned start of the seasonal calving period for each herd. The association between the proportion of heifers with clinical mastitis and all variables from the initial prospective survey were initially tested using bivariate analysis (i.e. generalised linear models for continuous variables and chi-squared test for categorical variables). Some variables were transformed or converted from continuous to categorical variables. For example, herd-owners used a number of different pre-weaning calf nutritional programmes. To facilitate analysis, these were reduced to two dichotomous variables (i.e. was milk containing antibiotic fed to calves (yes/no); and was colostrum fed to calves (yes/no)). Additionally the herd's total milk production (i.e. total kg milk solids (fat +protein) for the herd for the lactation) was converted to a cow level production variable (i.e. total milk solids production/total number of cows present in the previous lactation, kg/cow/lactation). Stocking rate of the herd (i.e. cows/Ha) was calculated by dividing the total number of cows and heifers present at the start of lactation by the effective farm area (Ha). Where continuous variables were not normally distributed, a new variable consisting of two or four categories were created. Correlations between numerical variables were tested using Pearson's correlation, between ordinal variables using the gamma correlation statistic. If two variables were significantly correlated (i.e. correlation >0.8), then the most biologically plausible variable was tested and the other excluded from the subsequent analysis.

Variables identified as associated (i.e. $p<0.3$) with proportion of heifers with clinical mastitis in the initial bivariate analysis were tested in a manual stepwise forward quasi-binomial generalised linear model. A quasi-binomial model differs from a binomial model only in that the dispersion parameter is not fixed at one and therefore it can model for over-dispersion. Variables with $p<0.3$ from the bivariate analysis were offered to the model rather than the more commonly used $p<0.25$, as survey data is often cruder, and so to minimise the risk of missing possible associations the larger p value was used. In this step-wise procedure the effect of each variable on the residual deviance of the model was evaluated but the criteria for a variable to remain in the model was $p<0.05$. Region was forced into the model, as it was a design-blocking variable. Where a variable was potentially either a continuous or categorical variable, the coefficients were graphed, and the deviance of the models containing the same data in continuous and categorical form were compared and the model with the best fit was selected based on lower deviance and larger error terms. Chi-squared tests of the deviances were used to compare the difference between the models, in order to select the final model of best fit. The first order interactions between significant main effects that were biologically plausible were examined. However, there were no significant interaction terms in the final model. The model goodness of fit was tested using Cooks statistic and plotting outliers (data from which studentised residuals were >SD2). A pseudo R^2 for the model was calculated using the null and residual deviances.

Using the final multivariate model, the predicted proportions of heifers in a herd with clinical mastitis were calculated for varying average milk solids/cow/lactation/herd and for the categorical variable herd clinical mastitis percentage and for the number of cows milked by each person within a herd. These predicted proportions were plotted against the variable of interest with a line of best fit drawn through the predicted values. The coefficients, calculated odds ratios (OR) and 95% confidence intervals (95%CI) for all the variables from the final multivariate model were also reported (Table 4). An OR>1 indicates an increased chance of clinical mastitis, while an OR<1 indicates a decreased chance. Although OR may overestimate the effects of the risk factors on the outcome tested when the disease is common (Dohoo et al 2003, pg 125-6; McNutt et al 2003), in this study they were deemed an acceptable approximation of the effects of the risk factors tested.

Statistical analysis carried out using "R: A language and environment for statistical computing" (R Development Core Team, 2005, www.r-org.org) version 1.9.

Results

A total of 578 of 926 (62%) herd-owners/managers responded to the initial prospective survey (99.5% of EDVS clients and 51.0% of AHC clients). Of these herds 272 (47.1%) returned clinical mastitis data. Of the 272 survey responses with clinical mastitis data, 250 provided sufficient data for analysis and the herd-owners had remained in milk production for the entire season (173 of 362 or 47.8% were AHC clients and 77 of 216 or 35.6% were EDVS clients). The PSC of the 250 herds ranged between the 10th June 2003 and the 18th August 2003 (mean=21 July, median=20 July). There was no difference in the results for any of the variables between the herds providing data from the initial survey (n=578) and the subset of herds providing clinical mastitis data (n=250).

The descriptive data for the herds is included in Table 2 (categorical variables) and Table 3 (continuous variables). The mean percentage of heifers within a herd was 17.8% (SD 3.8).

There were 1746 heifers recorded with clinical mastitis in the first 120 days of lactation. Of those heifers 1419 had clinical mastitis in only one quarter, 187 in two quarters, 22 in 3 quarters and 61 in all four quarters. There were 67 heifers in which the quarter was not recorded. The 1746 records were comprised of 1531 heifers. There were 1344 heifers with one case of mastitis, 163 heifers with 2 cases of mastitis, 21 heifers with 3 cases of mastitis, 2 heifers with 4 cases of mastitis, and one heifer with 5 cases of clinical mastitis recorded over the 120-day period. The average number of cases recorded per heifer was 1.14.

The median proportion of a herd with clinical mastitis in the first 120 days of lactation after the PSC was 11.95% (SD = 10.6, Range 0-57.8%, Quartiles 5.26%, 11.95%, 20.0%) of heifers and 9.0% (SD 6.3, Range 0-35.1%, Quartiles 4.63%, 7.63%, 12.82%) of cows (Appendix 2). There was a higher proportion of clinical mastitis in heifers than cows in July and August (5.5% vs. 1.2%, for July; 7.0% vs. 3.4%, for August, $p<0.001$), and a higher proportion of clinical mastitis in cows than heifers in September, October and November ($p<0.001$; Figure 2).

Bivariate analysis
The bivariate relationships between the management variables and the proportion of heifer clinical mastitis are presented in Table 2 (categorical variables) and Table 3 (continuous variables).

Variables associated ($p<0.3$) with the proportion of heifers with clinical mastitis at the bivariate level included keeping heifers on the main farm, training heifers in the milking parlour before calving, removing heifer's tails, application of teat antiseptic, managing lactating cows in more than one group, creating a separate management group based on expected calving date over the non lactating period, feeding milk from cows with mastitis to calves, feeding colostrum to calves, total milk solids production for the farm, individual cow milk production, the number of staff milking cows, the ratio of cows per staff member milking, the number of times heifers were trained through the milking parlour before calving, and the proportion of clinical mastitis in the cows (Table 2 and 3).

Multivariate analysis
The proportion of the herd with clinical mastitis within the first 120 days of lactation in heifers increased with increasing clinical mastitis in multiparous cows in the herd (Figure 3; $p=0.04$), increasing number of cows milked per person (Figure 4; $p=0.003$), increasing milk solids production per cow (Figure 5; $p<0.001$), and with increasing number of cows per hectare of farm area ('stocking rate'; $p=0.002$). The proportion of heifer mastitis was lower in herds where the milking cows were managed in more than one group (Table 4; $p=0.017$).

The pseudo R^2 for the final model was 0.31, which indicates that 31% of the variation around the proportion of heifers in a herd with clinical mastitis was accounted for by the variables in this model.

Discussion

The proportion of dairy heifers with clinical mastitis within a herd was shown to increase with higher herd production, higher proportion of cows with clinical mastitis, higher number of cows/person milking, higher stocking rate and management of the lactating cows in one group compared to multiple groups.

The study design was both a survey (descriptive) with the aim of identifying an estimate of the proportion of heifers in a herd with clinical mastitis over the first 120 days of lactation, and a fixed cohort observational study (number of animals were fairly constant over 120 days), where risk factors (exposures) were recorded prior to the commencement of the possible recording period for disease (outcome; Dohoo et al 2003, pg 151-61). The only risk factor not recorded prior to the outcome of interest was proportion of clinical mastitis in the cows. As the exposure factors occurred and were recorded prior to the outcome of interest, concerns about reverse causality are unlikely. For example, increased herd production/cow for the previous season was associated with increased proportion of heifers with clinical mastitis, and the alternative order of the events is biologically implausible.

A survey approach was used as a rapid and cost-effective way to provide an estimate of the proportion of clinical mastitis in the heifers and to examine a large number of potential herd level variables associated with the risk of clinical mastitis in heifers. However, surveys have some limitations such as demonstrating associations rather

than 'cause and effect', and may have some biases due to a failure for all contacted herd-owners to reply. To reduce the risk of biased estimates of effects (due to non reply), data from surveys may be weighted using expansion weights (Dargatz and Hill 1996). The expansion weight can also be thought of as the interval between sampled units under a systemic selection procedure or the number of populations represented by the sampled unit. To weight surveys there is a need for some *a priori* estimate of prevalence of the variables of interest and risk factors in the population. However, no *a priori* data were available and hence the survey responses were not weighted based on proportion of surveys returned.

Only two dairying regions in New Zealand were surveyed, which may limit the generalisation of the inferences made in the current study to a national level. However, the survey population characteristics were similar (within two SDs) to nationally reported values (Anon 2004). The average herd size and SD of the farms that responded to the survey was 290 cows (SD 150; national average 302), farm size was 77.9 Ha (SD 36.6; national average 111), the stocking rate was 3.7 cows/Ha (SD 0.6; national average 2.75) and the estimated average production per cow was 270.2 KgMS/lactation (SD 44.1; national average 322 kg). A possible explanation for the higher stocking rate and lower per cow production compare to the national average, is associated with how they were calculated. Herd size was calculated from the sum of cows and heifers. However some herd-owners when filling in the questionnaire may have interpreted the total number of cows inclusive of heifers, therefore artificially inflating the calculated herd size. Although only two regions were surveyed, there is moderate external validity of the data, suggesting that the inferences are likely to be of some value to other regions in New Zealand.

The response rate of the descriptive survey was 62%, which is an acceptable response rate for a mail out survey. However, the response rate for the clinical mastitis data was only 47% of those that responded to the descriptive survey. This was lower than expected and intended, and created a potential bias. However, there was no difference in the descriptive variables between the herds used in the final data analysis and those in the initial descriptive survey. This implies that generalisations from the analysis can be applied to the entire survey population with some confidence.

Another potential source of bias in this study was that the herd-owner undertook both the diagnosis and recording of clinical mastitis cases. Part of the observed herd variation in prevalence may be related to differences in sensitivity and specificity of diagnosis of clinical mastitis and thoroughness of recording.

The cumulative incidence of heifer mastitis in this study was 13.6% (SD 10.6) and the cumulative incidence of mastitis in multiparous cows was 9.0% (SD 6.3) in the first 120 days of lactation after the PSC. Others have demonstrated that there is a peak in incidence of heifer clinical mastitis around the time of calving (Barkema et al 1998), which is supported by the temporal distribution of cases of clinical mastitis in this survey matching the seasonal calving pattern shown (Figure 2). The prevalence of heifer mastitis, of 13.6%, is higher than found in previous New Zealand studies where a cumulative incidence in heifers in the first five days post-calving was 8.1% (Pankey et al 1996) and 9.9% across all parities in the first six weeks of lactation (McDougall 1998). Comparatively, lactational incidences of clinical mastitis for all parities of cows from Northern Hemisphere studies covered a wide range between 3% and 34% (Myllys and Rautala 1995; Edinger et al 2000). Possible reasons for differences in cumulative incidence estimates between these studies and the current study include

the herds selected to participate in the studies, annual seasonal variation and variation in the diagnostic criteria for clinical mastitis.

Another possible explanation for an inflated estimate of heifer clinical mastitis prevalence in the current study is the inclusion of multiple cases for each animal where the farmer recorded them. Repeat cases due to treatment failure or new infection occurred in 12.6% of quarters. Final multivariate analysis was performed using the data with repeat cases excluded (true binomial model) and it was compared to the final multivariate model with repeat cases included. There was no difference in the effects of the risk factors on heifer clinical mastitis prevalence in these two models; therefore the repeat cases did not bias the data in any way. Modelling the data including the repeat cases could have been done using a poisson distribution of the outcome, (multiple counts/cow). As calving date was not recorded for each animal, time at risk for each animal could not be calculated and therefore incidence rates could not be estimated. The outcome presented is therefore the proportion of heifers with clinical mastitis over 120 days.

There was a significantly higher proportion of heifers with clinical mastitis compared to multiparous cows in July and August. In September, October and November this is reversed. The proportion of heifers calving closer to the PSC and therefore leading to an apparent higher proportion of clinical mastitis during this period may be confounding this. However, the different temporal patterns of distribution of heifer vs. cow clinical mastitis across lactation could also be associated with other factors such as; an increase in contagious pathogens causing clinical mastitis later in lactation (McDougall 2003), increased susceptibility to contagious pathogens in older cows with previous history of clinical mastitis and high SCCs (McDougall, unpublished) and teat end/teat canal damage (Elbers et al 1998). The trend in the current study supports the results of previous studies that show a greater increase in SCC toward the end of the lactation with higher parity cows with the SCC curve being relatively flat in heifers (Schepers et al 1997). The pathogens causing clinical mastitis in early lactation are primarily environmental pathogens, such as *Streptococcus uberis*, which is the primary cause of clinical mastitis in heifers in New Zealand (Pankey et al 1996; McDougall 1999).

Heifers are the largest parity group in the New Zealand dairy herd structure (17.8% in this study). They are the animals in the herd with the greatest genetic potential and are an expensive resource in terms of input up until the point of calving, and before calving they are yet to contribute to the financial position of the herd-owner. Therefore, mastitis in heifers is potentially the one of the most costly diseases for farmers.

A number of risk factors were identified using bivariate analysis, which were not significant in the multivariate model when confounding effects of other factors were adjusted for (Dohoo et al 2003, pp. 235-270). However, the bivariate analysis has also been reported so that readers are able to draw their own conclusions as to the importance of these variables. These variables form a basis for discussion about other possible risk factors not identified in the multivariate model.

Feeding of antibiotic milk was associated with an increased proportion of heifers with clinical mastitis. It has been hypothesised that feeding calves milk from cows with clinical mastitis may result in transmission either mechanically due to cross-suckling (Kesler 1981) or due to haematogenous spread to the mammary gland following

absorption of ingested pathogens. The literature is not conclusive and further work needs to be done to identify if and how mastitic milk is a risk to heifers (Yagi et al 2004).

Grazing calves or heifers on the same farm area as the milking cows, rather than away from the milking cows was associated with a decreased proportion of heifers with clinical mastitis. Grazing the calves away from the milking herd may increase exposure to mastitis pathogens through transport, exposure to other calves and cows or increased stress resulting in depression of immunity (Fox et al 1995). Heifers generally return to the home farm 6 to 12 weeks before calving, which suggests that infection may be acquired during or before this time. Intramammary infections have been isolated in animals as young as nine months old (Trinidad et al 1990), supporting the hypothesis that infection is occurring well before first parturition.

Training heifers through the milking parlour before calving was associated with an increased the proportion of heifers with clinical mastitis. This may be associated with increased exposure to mastitis pathogens due to walking the heifers to and from the milking shed, which may increase exposure to environmental pathogens that are present in large numbers on races, in gateways and on dirty milking yards (Leigh 1999). Alternatively, increasing stress in heifers prior to calving, due to extra movement may suppress the immune system and increase their susceptibility to infection (Mallard et al 1998). Conversely, training the heifers may reflect a more intensive level of management in some herds, including higher sensitivity to diagnose clinical mastitis.

Tail removal was associated with a decreased proportion of heifers with clinical mastitis. This may be associated with improved udder hygiene, as pre-milking udder hygiene is associated with milk quality (Pankey et al 1987; Schreiner and Ruegg 2003). However, a number of studies have shown that the cleanliness of the udder is not improved with tail removal (Eicher et al 2001; Tucker et al 2001; Schreiner and Ruegg 2002). Furthermore these studies showed no difference in SCC and IMI in docked compared to undocked animals (Tucker et al 2001; Schreiner and Ruegg 2002). Whether a herd tail-docked their heifers was not a significant variable in the multivariate analysis, which suggests that the herds that tail-dock their heifers may use other management practices that are associated with lower cumulative incidence of clinical mastitis in heifers, or that the practice of tail-docking may be confounded by herd size or some other covariate that remained in the final model.

Routine manual stripping of the mammary gland for all heifers within four days of calving was associated with a higher proportion of heifers with clinical mastitis heifers. This may be a result of increased sensitivity of detection of mastitis during the colostrum period associated with stripping. Alternatively, herds with a history of high incidence of clinical mastitis or with elevated BTSCC may be more likely to undertake increased levels of surveillance for mastitis, including regular stripping of quarters within the colostrum period.

The final multivariate model identified several factors significantly associated with the proportion of heifers in a herd with clinical mastitis. A high average per cow milk production, a high number of cows milked per person, a high percentage of cows with mastitis in the herd (as a categorical variable in quartiles), lactating cows managed in only one group and high stocking rate (as a categorical variable > or <3.3cows/ha) were associated with a higher proportion of heifers with clinical mastitis in a herd.

Herds with higher milk solids production per cow per season were more likely to have a higher proportion of heifers with clinical mastitis. This is consistent with other studies that have found higher herd level production is associated with an increased incidence of clinical mastitis (Schukken et al 1990; Grohn et al 1995; Rupp and Boichard 2000). However, this is in contrast to the findings that herds with higher production had lower BTSCC and better udder health compared to herds with lower production (Wilson et al 1997). One explanation for this apparent contradiction could be that despite the increased risk of clinical mastitis associated with higher production, herds with very high levels of production are aware of the effects of SCC on production, and to reach these higher levels of production better management practices are required, such as earlier detection and treatment of clinical and/or subclinical mastitis, leading to earlier detection and removal of the offending cows from milk supply. This increase in surveillance may in fact inflate the incidence of clinical mastitis in these herds with high BTSCCs.

Cow level factors that are associated with higher production, such as increased dripping of milk and udder oedema pre-calving, have also been shown to increase the risk of clinical mastitis (Schukken et al 1990; Slettbakk et al 1995; Waage et al 1998).

Herds with stocking rates >3.3 cows/ha compared to herds with lower stocking rates had an increased proportion of heifers with clinical mastitis. Increasing stocking rate may be associated with increased environmental exposure to mastitis pathogens, reduced nutrient intake, or an increase in the between cow antagonistic interactions, all of which may increase the risk of mastitis. Poor hygiene in dry period housing or faecal contamination of bedding around calving are significant risk factors for environmental mastitis (Smith et al 1985; Todhunter et al 1995). Additionally, cows with poor udder hygiene have been demonstrated to have higher SCC and increased prevalence of intramammary infection (Schreiner and Ruegg 2003). Cow hygiene is difficult to manage in pasture-based systems, especially during the peripartum period. Few herd-owners have facilities such as 'stand-off pads' or barns that can be used in periods of adverse weather, and those that are available may not be adequately cleaned. Further work is required to identify the mechanisms by which stocking rate affects the risk of clinical mastitis and to investigate management strategies to mitigate the effects of high stocking rates.

Managing lactating cows in more than one group was associated with a lower proportion of heifers with clinical mastitis. This may be due to the reduction in spread of contagious pathogens from cows to heifers, or it may be associated with a reduction in the between cows and heifer antagonistic interactions, which may induce stress and associated immunosuppression (Barnouin and Chassagne 1998; Mallard et al 1998; Phillips and Rind 2001). In the current study, age was the most common basis for allocation of cows to management groups. Additionally, the proportion of heifers with clinical mastitis had a strong positive association with the proportion of cows with clinical mastitis in a herd. This is may be due to the increased risk of spread of contagious pathogens but may also indicate that herds with high incidence of clinical mastitis in their cows have generally poor mastitis management systems for both cows and heifers (Barkema et al 1999b). Factors that have not been captured in the current survey such as hygiene of the races, milking machine management and hygiene during the milking procedure, have been identified previously as risk factors for the incidence of clinical mastitis (Smith 1983; Pankey 1989; Spencer 1989; Pankey et al 1994).

Increasing the number of cows milked by one person was associated with an increase in the proportion of heifers with clinical mastitis in that herd. This may be due to a reduced level of conscientiousness or reduced thoroughness of the milking routine. For example, clusters may be left on cows for too long and result in over-milking. It has been demonstrated that 'slow and thorough' milkers are associated with lower BTSCC than in herds milked by 'quick and dirty' milkers (Barkema et al 1999b). The increase in risk of mastitis with increasing numbers of cows/person may appear paradoxical as reduced sensitivity of detection of mastitis might be expected to occur when large numbers of cows are milked by one operator.

Conclusions

This study provided estimates of the prevalence of various heifer mastitis-related management strategies in two regions of New Zealand, and identified some herd level risk factors for heifer mastitis in pasture-based systems. The proportion of dairy heifers with clinical mastitis was found to be 13.6%. The proportion of heifers with clinical mastitis in a herd increased with increasing stocking rate, managing lactating cows in a single group, increasing the number of cows for each person milking, increasing the proportion of cows with clinical mastitis in the herd and an increasing the per cow production. While not proving causation, this study has identified risk factors, which require further detailed and controlled studies to identify their mechanism of action and prove causality.

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Figure 2 The mean (SEM) herd (n=250 herds) proportion of heifers (grey) and cows (white) diagnosed with lactational clinical mastitis from calving to approximately 120 days into lactation for each calendar month. The proportion of cases of clinical mastitis/herd for cows and heifers was compared using chi-squared analysis, the significantly different proportions are indicated * ($p<0.05$).

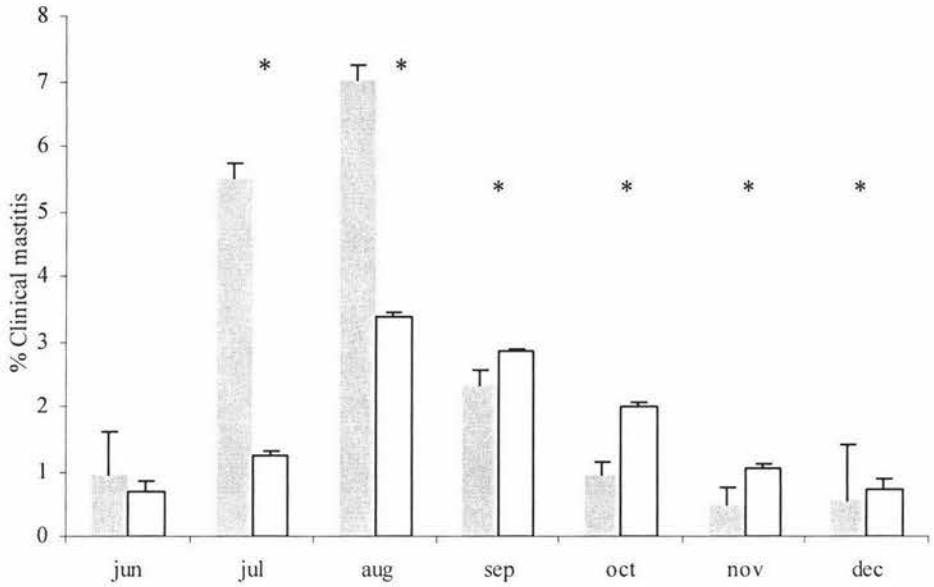

Figure 3 Predicted proportions (SEM) of heifers with clinical mastitis over the first 120 days of lactation for ranges of percentage of cows in a herd with clinical mastitis during the first 120 days of lactation. The predicted proportions were adjusted for mean production of milk solids (i.e. 270 kg MS /cow/lactation), mean number of cows per person milking (i.e. 170 cows/person), single milking mob and in the Waikato region. The trendline represents linear line of best fit.

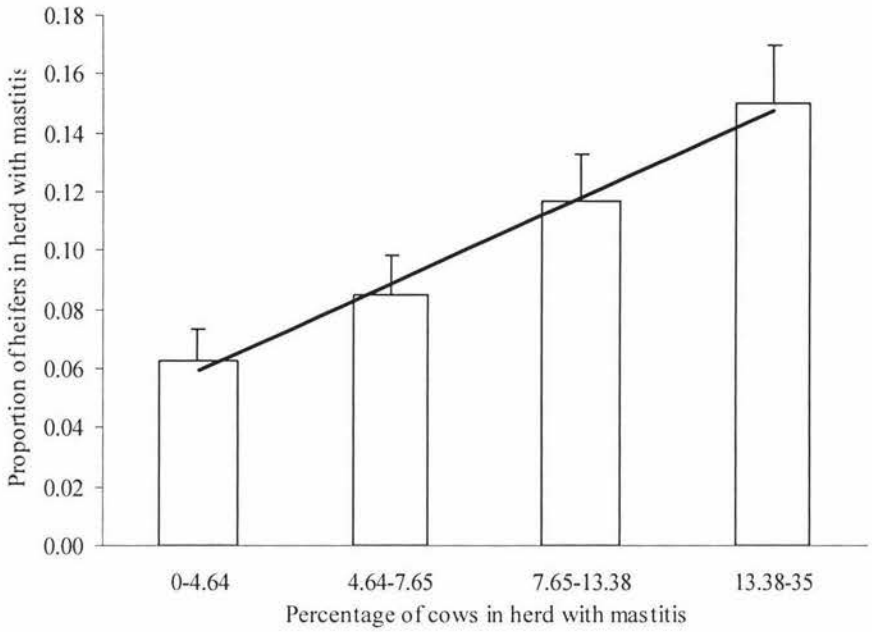

Figure 4 Predicted proportions (SEM) of heifers with clinical mastitis over the first 120 days of lactation for the average number of cows per person milking. The predicted proportions were adjusted for mean production of milk solids (i.e. 270 kg MS/cow/lactation), mean herd proportion of cows with clinical mastitis of 7.7-13.4%, single milking mob and in the region Waikato. The trendline is the linear line of best fit for the model.

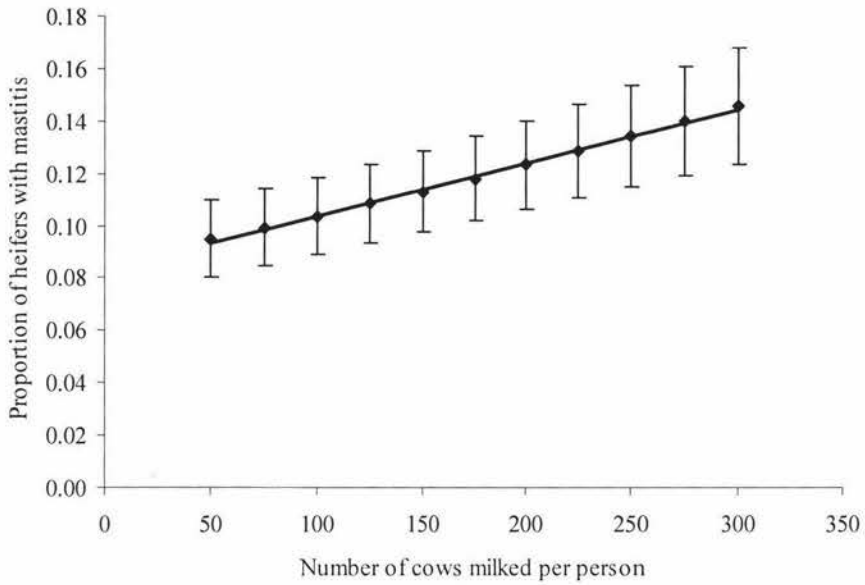

Figure 5 Predicted proportions (SEM) of heifers with clinical mastitis over the first 120 days of lactation for average milk production per herd (i.e. kg milk solids/cow/lactation calculated using farm production for the 2002/2003 season/number of cows at the start of the 2003/2004 season). The predicted proportions were adjusted for mean herd proportion of cows with clinical mastitis of 7.7-13.4%, average number of cows/person milking (i.e. 170 cows/milkers), a single milking mob and in the region Waikato. The trendline is the linear line of best fit from the model.

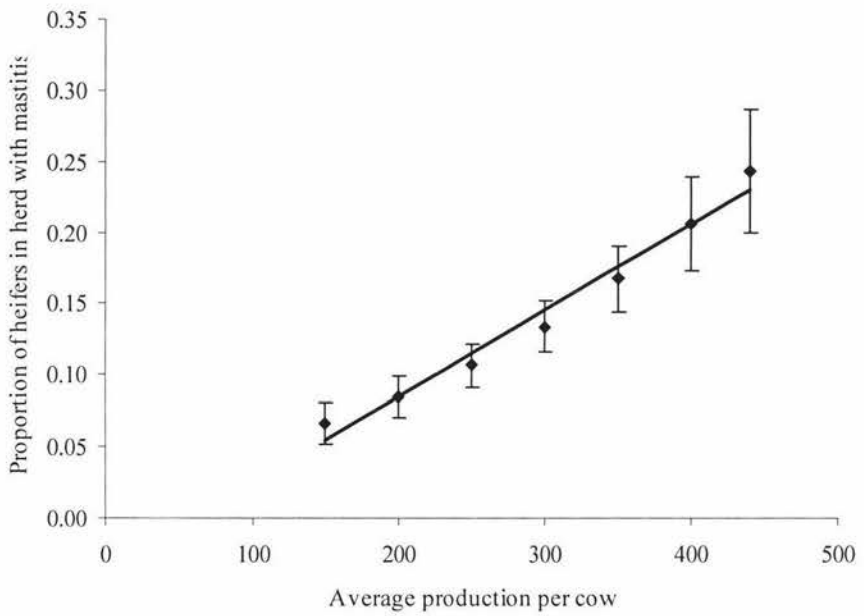

Table 2 Number of herds (n=250) in which potential risk factors (variables) for clinical mastitis in each level for the categorical variables, the bivariate odds ratio (OR) and bivariate probability (P) that that variable was associated with the proportion of heifers in a herd with clinical mastitis

Variable Name	Description of Variable	Levels	n	OR	P
springers	Is a 'point of calving' separated before calving?	NO	81		ref
		YES	169	1.01	0.92
springmgt	How long before calving are the cows drafted to this group?	<1 week before due	29		ref
		1-2 weeks before due	87	1.09	0.6
		>2 weeks before due	45	0.91	0.67
		other	5	0.47	0.19
colost	Is a separate colostrum group run?	NO	45		ref
		YES	205	0.88	0.43
multmilkers	Are there multiple milking herds?	NO	195		ref
		YES	55	0.86	0.22
milkersplit	Basis on which the milking herd divided	Age	31		ref
		Body Condition	8	0.75	0.42
		Calving Date	8	1.84	0.03
		Other	8	0.65	0.247
multdrys	Are there multiple groups through the dry period?	NO	48		ref
		YES	202	1.02	0.93
drysplit	Basis on which the dry herd is divided ?	Age	77		ref
		Age & Body Condition	18	1.06	0.80
		Body Condition	83	1.17	0.25
		Calving Date	12	1.15	0.59
		other	12	0.9	0.72
springoff	Is the pre-calving group taken off pasture and stood on concrete/tracks if it gets very wet?	NO	146		ref
		YES	104	1.08	0.47
replacesource	Where are the replacement stock from?	Bred at Home	239		ref
		Purchased	2	0.44	0.21
		Both	8	1.05	0.87
ab	Were the calves fed milk that was withheld from the vat due to antibiotic contamination?	NO	149		ref
		YES	101	1.17	0.16
colostrum	Were the calves (once >4days old) fed colostrum (milk from <4 days after calving)?	NO	23		ref
		YES	227	1.25	0.22
calfgrazed	Where are the calves grazed from 3-12months? Grazier = farmer pays a fee to a land owner for growing out their stock, Home = on the dairy farm, Runoff = farmer owns the land and stock are grazed on it but it is separate to dairy farm	Graziers	33		ref
		Home	124	0.78	0.12
		Runoff	70	0.89	0.49
		Both	23	0.85	0.47
calfgrazebhcows	If the calves are grazed on the dairy farm they may graze the land the cows have been grazing immediately after the cows are moved on.	NO	236		ref
		YES	14	0.81	0.35

graze12mthon	Where are the heifers grazed from >12months? Grazier = farmer pays a fee to a land owner for growing out their stock, Home = on the dairy farm, Runoff = farmer owns the land and stock are grazed on it but it is separate to dairy farm	Graziers	181		ref
		Home	20	0.73	0.24
		Runoff	48	1.05	0.72
		Both	1	1.01	0.99
hfrrunshed	Are the heifers trained by walking them through the milking parlour before calving?	NO	84		ref
		YES	166	1.16	0.20
hfrspraytrain	If the heifers are trained are they teat sprayed during training?	NO	224		ref
		YES	26	0.97	0.85
hfrmastchecktrain	If the heifers are trained are they checked for mastitis during training?	NO	195		ref
		YES	55	1.01	0.95
hfrdock	Are the heifer's tails docked?	NO	164		ref
		YES	86	0.86	0.18
hfrprecalv	When do heifers join the main herd?	Remain own herd throughout the season	2		ref
		Join the main herd <4weeks before calving	20	1.48	0.60
		Join the main herd after calving	82	1.64	0.49
		Join the main herd >4weeks before calving	135	1.79	0.41
		Join into a second herd before calving	11	1.64	0.51
oxyallhfr	Use oxytocin in all heifers routinely	NO	240		ref
		YES	10	1.33	0.25
oxysomehfr	Use oxytocin in heifers that don't let milk down routinely	NO	112		ref
		YES	138	1.09	0.45
teatspray		Don't teat spray	16		ref
		Post milking teat spray	203	2.4	0.01
		Pre milking teat spray	7	1.98	0.18
		Pre and post milking	24	2.26	0.04
rxsubclincals	Are cows treated for subclinical mastitis?	NEVER	158		ref
		SOMETIMES	92	0.92	0.49
coloststrip	How often are the cows in the colostrum period stripped to check for mastitis?	at first milking	31		ref
		first and last milking	90	1.11	0.59
		once daily	45	1.07	0.76
		other	2	0.6	0.52
		twice daily	82	1.53	0.03
hfrmastprob	Farmers were asked if they perceived heifer mastitis as being a problem	NO	171		ref
		YES	79	1.56	<0.001

Table 3 Descriptions (mean, standard deviation (Std Dev), minimum (min), maximum (max) and number of herds (n)), bivariate odds ratio (OR) and bivariate probability (p) for potential risk factors that were continuous variables for the proportion of heifers with clinical mastitis.

Description of Variable	n	Mean	Std Dev	Min	Max	OR	p
Effective farm area (Ha)	247	77.9	36.6	11.0	290.0	1.00	0.78
Stocking rate (cows/Ha)	247	3.7	0.6	1.6	5.5	1.04	0.66
Cows in herd at start of season	250	238.8	126.1	29.0	1000	1.00	0.87
Heifers in herd at start of season	250	51.3	26.9	7.0	180.0	1.00	0.78
No. of days difference between PSC[y] for heifers and cows	250	1.2	3.4	-20.0	13.0	1.00	0.79
Farm production for 2002/2003 season (Kg milk solids)	241	76 901	36 535	7 500	270 000	1.00	0.11
Milk solids per animal in 2002/2003 season (Kg milk solids)[x]	241	270.2	44.1	118.7	439.7	1.01	<0.001
No. of staff routinely milking	250	1.7	0.6	1.0	3.0	0.83	0.05
No. of animals in herd/No. of milkers	250	170.1	69.0	31.0	490.0	1.00	0.06
No. of times the heifers are trained through the milking shed before calving	249	3.0	3.6	0.0	30.0	1.03	0.05
Percentage of cows with clinical mastitis out of total no. of cows at start of season[z]	250	9.0	6.3	0.0	35.1	1.05	<0.001

[x] calculated using the total number of cows at the start of the 2003/2004 season

[y] PSC Planned start of calving

[z] not normally distributed, included transformed to a categorical variable

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) with probability values (p) calculated from the multivariate final model for the outcome variable proportion of heifers with clinical mastitis in a herd.

				95% CI		
Variables	Coefficient	Std Error	OR	Upper	Lower	P
(Intercept)	-4.47	0.38				
Milk production (kg fat+protein/cow/lactation)	0.01	0.00	1.01	1.01	1.00	<0.001
No. cows/person milking	0.002	0.001	1.002	1.003	1.001	0.003
Incidence of clinical mastitis in cows (%/120 days)						
<4.64 (63 herds)	ref					
4.64 to 7.65 (63 herds)	0.33	0.16	1.39	1.89	1.01	0.04
7.65 to 13.38 (66 herds)	0.68	0.15	1.98	2.64	1.48	<0.001
>13.38 (65 herds)	0.97	0.15	2.63	3.52	1.97	<0.001
Milking cows managed in multiple groups:						
No (195 herds)	ref					
Yes (55 herds)	-0.27	0.11	0.76	0.95	0.61	0.02
Stocking rate (cows/Ha)						
<3.30 cows/Ha (60 herds)	ref					
>3.30 cows/Ha (187 herds)	0.43	0.14	1.54	2.02	1.17	0.002
Region						
Taranaki (77 herds)	ref					
Waikato (173 herds)	0.03	0.12	1.03	1.31	0.81	0.83

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Chapter 4 Subclinical and clinical mastitis in heifers following the use of a teat sealant pre-calving

Abstract

This study investigated the effect in heifers of infusion of a bismuth subnitrate teat-canal sealant and bacterial intramammary infection (IMI) pre-calving, on prevalence of post-calving IMI and incidence of clinical mastitis in the first two weeks post-calving. Glands (n=1020) from heifers (n=255) in five seasonally calving, pasture-fed dairy herds were randomly assigned within heifer to one of four treatment groups (no treatment; mammary gland secretion collection; infusion of a teat sealant; or sample collection with infusion of teat sealant). Heifers within a herd were enrolled on one calendar day, on average 31 days before the planned start of the seasonal calving period. Duplicate milk samples were collected from each gland within four days after calving for bacterial culture. Herd-owners collected duplicate milk samples, before treatment, for bacterial culture from glands they defined as having clinical mastitis. The relative risk of post-calving infection and clinical mastitis were calculated using Mantel Haenszel or logistic regression analyses. The gland prevalence of IMI pre-calving was 15.5% and did not differ between herds. Bacteria isolated pre-calving included coagulase-negative staphylococcus (76.9% of all bacteriologically positive samples), *Streptococcus uberis* (14.1%), *Staphylococcus aureus* (5.1%), *Corynebacterium* spp. (3.8%), and others (0.1%). The presence of an IMI pre-calving increased the risk of an IMI post-calving 3.5 times and the risk of clinical mastitis 4 times, relative to no IMI pre-calving. Infusion of the teat sealant reduced the risk of post-calving IMI due to *Streptococcus uberis* by 84%, and of clinical mastitis by 69%. Sampling the glands pre-calving had no effect on post-calving IMI or on clinical mastitis incidence. Use of an internal teat canal sealant in heifers pre-calving reduced the prevalence of IMI and clinical mastitis post-calving, and provides a useful tool for reducing the risk of subclinical and clinical mastitis in heifers.

(***Keywords***: mastitis, *Streptococcus uberis*, heifer, teat sealant)

***Abbreviation key*: IMI** = intramammary infection; **RR** = relative risk; **C** = control; **S** = sample; **TS** = teat sealant; **ICC** = intraclass correlation

Introduction

Peripartum clinical mastitis incidence is greater in heifers than in older herd mates (Barkema et al., 1998). The epidemiology of mastitis peripartum may be different in heifers from cows, as heifers have not been exposed to the milking process. Risk factors for intramammary infections (IMI) or clinical mastitis in heifers are not well defined in pasture-based dairy systems and specific recommendations for control of mastitis in heifers are not available. Current mastitis control programmes do not provide specific advice about managing heifer mastitis.

Intramammary infection and clinical mastitis in heifers in early lactation results in long-term production loss (Woolford et al., 1984), an increased risk of clinical mastitis in the subsequent lactation and of premature removal from the herd (Rupp et al., 2000; Waage et al., 2000).

Bacteria have been isolated from the mammary gland of heifers as early as 9 months pre-calving (Trinidad et al., 1990a). Prevalence of infection increases as calving approaches (Oliver and Mitchell, 1983; Aarestrup and Jensen, 1997). Estimates of prevalence pre-calving range from 20% to 97% (Meaney, 1981; Trinidad et al., 1990a; Myllys, 1995). The peripartum period is associated with increased risk of new IMI and clinical mastitis relative to earlier in gestation, likely to be associated with the transition into lactation (Aarestrup and Jensen, 1997).

In intensive dairy systems the bacteria isolated from the mammary gland of heifers' peripartum include *Staphylococcus aureus*, coagulase-negative staphylococcus, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Corynebacterium* spp. and coliforms (Oliver and Mitchell, 1983; Trinidad et al., 1990a; Fox et al., 1995). Environmental factors affect the type and prevalence of bacterial pathogens associated with bovine mastitis. Infection with environmental *Streptococcus* spp. occurs most commonly during the dry period and in the first month of lactation (Todhunter et al., 1995; Leigh, 1999). *Streptococcus uberis* is the most common cause of clinical mastitis in the pasture-based management systems in New Zealand (Williamson et al., 1995; Pankey et al., 1996; McDougall, 1999).

Presence of *Streptococcus dysgalactiae* IMI pre-calving increases the risk of clinical mastitis post-calving in heifers (Aarestrup and Jensen, 1997). However, in lactating cows, pre-existing infection with a minor pathogen may reduce the risk of subsequent major pathogen infection (Lam et al., 1997).

Intramammary infusion of antibiotics pre-calving in heifers has been shown to reduce the prevalence of IMI and to reduce the incidence of clinical mastitis post-calving (Oliver et al., 2003) by removing existing infections and reducing the risk of new infections. However, use of antibiotics may increase the risk of residues in milk and new infections may still be acquired before calving, as the period over which the antibiotics remain above the minimum inhibitory concentration in the glands is highly variable between heifers (Trinidad et al., 1990b; Oliver et al., 1992). Infusion of a teat sealant at the end of lactation has been shown to reduce the new IMI rate over the non-lactation period in multiparous cows (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002) by acting as a physical barrier within the teat canal over this period. Use of a teat sealant reduces the risk of antibiotic residues in milk and most likely remains in place until it is physically removed from the gland.

It was hypothesised that infusion of a teat sealant before first calving would reduce the prevalence of IMI post-calving and the incidence of clinical mastitis postpartum, and that the reduction would be greatest for the *Streptococcus* spp.
The objectives of this study were to determine:
1) The prevalence of IMI and to define bacterial species associated with IMI in pasture-fed heifers pre-calving;
2) The relationship between presence of IMI pre-calving and the probability of IMI post-calving and clinical mastitis;
3) The effects of sampling of the gland pre-calving on the risk of post-calving IMI;
4) The effects of infusion of a teat sealant pre-calving on the reduction in the incidence of new IMI with any bacterial species;
5) The effects of infusion of a teat sealant pre-calving on the reduction of prevalence of post-calving IMI with any bacterial species and specifically with *Streptococcus uberis;*
6) The effects of infusion of a teat sealant pre-calving on the reduction of the incidence of clinical mastitis and specifically reduce the incidence of mastitis caused by *Staphylococcus* and *Streptococcus* spp.

Materials and methods

Glands (n=1020) from heifers (n=255) in five spring-calving, pasture-fed dairy herds were enrolled. Herds were selected on the basis that herd-owners maintained high quality records and agreed to be involved with the study. The herds were located in the Waikato (n=4) or Taranaki (n=1) regions of the North Island of New Zealand. Between 38 and 60 heifers were enrolled from each herd.

A sample size of 240 animals was calculated based on the assumption that the gland level prevalence of IMI post-calving would be 30% in the control group and 15% in the teat sealant treated group, using a 95% confidence limit (α=0.05) with 80% power (β=80%) (Hintze, 2001, NCSS and PASS).

Heifers in each herd were enrolled on one calendar day, on average 31 days (SD=15) before the start of the seasonal calving period. The heifers calved between 6 and 89 days after enrolment. Treatment was assigned within heifer in a 2 by 2 factorial arrangement. Four different randomisation patterns were created and these were applied randomly within sequentially presented groups of four heifers. The four patterns were designed to prevent the possibility that one treatment was allocated only to front or rear glands (Figure 6). The risk of clinical mastitis is higher in the rear than front glands (Barkema et al., 1997), which represented a potential bias if treatments were not applied equally across the front and rear glands. The glands either had a secretion collected from them only ("S"); a gland secretion collected and then infusion with 2.6g of bismuth subnitrate teat sealant; Teat Seal, Pfizer Animal Health NZ Ltd, Auckland, New Zealand ("S + TS"); were infused with the teat sealant only ("TS"); or were left as an unsampled and untreated control gland ("C"). Before sampling and/or infusion, each teat end was scrubbed with a cotton wool pledget moistened in 70% methanol. No secretion was discarded before collection of this sample due to the small volume of secretion present in most glands. Where no secretion could be collected from a gland (n=4), a cotton wool pledget swab (Copan Venturi Transystem, Copan, Corona, California) was vigorously scrubbed at the teat end and this swab was cultured. The tip of the teat sealant cannula was inserted approximately 3 mm into the teat canal for infusion. Following sampling and infusion,

0.5% effective iodine was applied by manual spraying to all teat ends. Samples were processed for bacteriology within 24 hours of collection following storage at 4°C.

Within 4 days of calving (range 0 to 4 days after calving; median=2 days), duplicate milk samples (~20 ml each) were collected from each gland for bacterial culture by trained technicians, following aseptic preparation of the teat end and discard of the first 3 strippings. One heifer was sampled 32 days after calving and was excluded from further analysis. Milk samples were stored at 4°C until processing, which occurred within 24 hours of collection. Herd-owners collected duplicate milk samples from glands diagnosed with clinical mastitis, before treatment, from 4 days before to 14 days after calving. Clinical mastitis was defined as presence of grossly visible changes (e.g. clots or blood) in the milk composition or swelling and/or pain in gland Milk samples from clinical mastitis cases were frozen at -20°C for up to 14 days on farm before being collected for processing.

Calving date data was retrieved from the national database (Livestock Improvement Corporation, Hamilton, New Zealand).

Bacteriology

Following gentle inversion at room temperature, 10 µl of milk was streaked onto a quadrant of a 5% sheep blood agar plate containing 0.1% esculin (Fort Richard, Auckland, New Zealand), and incubated at 37°C for 48 h. The genus of bacteria was provisionally determined on the basis of colony morphology, Gram stain, hemolysis pattern, catalase test and esculin reaction. Gram-positive, catalase-positive isolates were further tested using the tube coagulase test, and coagulase-positive isolates were defined as *Staphylococcus aureus* and coagulase-negative isolates as coagulase-negative staphylococcus. Gram-positive, catalase-negative isolates were CAMP tested and esculin-positive and CAMP-positive or negative isolates were defined as *Streptococcus uberis*, esculin-negative and CAMP-negative isolates as *Streptococcus dysgalactiae* and esculin-negative and CAMP-positive isolates as *Streptococcus agalactiae*. Gram-negative rods were sub-cultured on MacConkeys agar, had an oxidase test performed and were cultured in triple sugar iron agar and simmons citrate agar. Gram-negative rods that could be speciated were recorded at species level, 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.

A milk sample was defined as contaminated if >3 distinct colony types were isolated present. A gland was defined as infected where ≥3 of each of 1 to 3 colony morphology types were present. Major pathogens were defined as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis* and *Streptococcus dysgalactiae*. When two bacterial spp were isolated from a gland the major pathogen was the species used in the further analysis. There were only 13 glands with multiple isolates from all milk samples in this study. A new infection was defined as occurring where either there was no IMI pre-calving and an IMI was present post-calving, or where there was a different bacterial species present from the pre-calving to post-calving sample.

Statistical analysis

Descriptive statistics at heifer and gland level were calculated.

Dependent variables included the presence of post-calving IMI, post-calving IMI that was a major pathogen, post-calving IMI with *Streptococcus uberis*, new IMI, clinical

mastitis, and clinical mastitis from which any pathogen or only a major pathogen were isolated.

Independent (fixed) variables included pre-calving IMI, sampling of the gland, infusion of the teat sealant, herd, interval between infusion and calving (a continuous variable in days), number of days post-calving at sample collection (categorical) and days calved relative to the median calving date for each herd (continuous variable (positive and negative integer)). Herd was included as a fixed effect due to the small number of herds (n=5), and as they were not randomly chosen.

Bivariate associations between independent and dependent variables were examined using chi-square analysis and the crude odds ratios (OR) were calculated. The Mantel-Haenszel procedure was used to stratify the data by herd and the adjusted relative risks (RR) were calculated. To test the hypothesis that sampling may have increased the risk of IMI post-calving or clinical mastitis, the number of glands with post-calving IMI and glands with clinical mastitis were compared between the glands that were sampled only pre-calving ("S") and the control glands ("C"), using chi-square analysis. Routinely where bivariate analyses did not identify significant relationships between the main effects (teat sealant, pre-calving IMI or sampling pre-calving) and the dependent variables, further multivariate analysis was not undertaken and bivariate results were reported (e.g. with new IMI and effect of sampling on outcomes). The main effects did not significantly affect clinical mastitis incidence in the bivariate analysis. However, because of concerns regarding the number of possible false positives in the teat sealant treated glands (see later in results and discussion) further analysis was undertaken for the outcome clinical mastitis from which a pathogen was isolated, or from which a major pathogen was isolated.

Predictor variables from the bivariate and stratified analyses associated with the outcome variables (i.e. $P<0.2$) were manually added in a stepwise manner to a logistic regression model (PROC GENMOD, SAS v 9.1, 2004). Variables were included where they reached significance ($P<0.05$). Biologically meaningful interactions between the main effects were tested including teat sealant by sampling, teat sealant by pre-calving IMI status, and herd by teat sealant.

The final models with post-calving IMI (any pathogen, major pathogen and *Streptococcus uberis*) as the dependant variable included herd, teat sealant, pre-calving IMI status and days from calving to sampling as fixed effects (Table 8; Models 1-3). The final models with clinical mastitis (clinical mastitis culturing any pathogen and clinical mastitis culturing a major pathogen) as the dependent variable included herd, teat sealant, pre-calving IMI status and days from calving relative to the median calving date of the group (Table 8; Models 4, 5). When pre-calving IMI was included in the models only half the glands were included in the analysis due to the 2X2 design of the experiment. Therefore, the same logistic regression models were tested without pre-calving IMI status included, which doubled the number of glands in the analysis (Table 8; Models 6-10). Clinical mastitis (including those cases not culturing pathogen) was only analysed at the univariate level, as the multivariate model was unstable.

The ORs were converted to adjusted RR (Beaudeau and Fourichon, 1998) as the OR may have led to an overestimation of the size of the main effects (Dohoo et al., 2003). Relative risks with 95% confidence intervals (CI) and number of glands (n) reported in the text were calculated from the final multivariate models.

Sixteen individual glands from 16 heifers were diagnosed with clinical mastitis and treated before the routine post-calving samples were collected. All glands from these animals were subsequently sampled at the routine visit within 4 days post-calving; no bacteria were cultured from any of these samples. The bacteriology result from the sample of the gland with clinical mastitis was recorded as the post-calving bacteriology result for that gland, and the bacteriology results from the remaining glands' samples collected at the routine visit within 4 days of calving, were recorded as the post-calving bacteriology result for those glands. To test the validity of this, a new binomial variable was calculated, treated before sampling (yes, no) and this variable was examined in all the logistic regression models. This variable and the interaction between sampling with treatment group were not significant in any model. Hence, the results from these 16 heifers (n=64 glands) remained in the final analysis, and the 'treated before sampling' variable did not remain in any of the final models.

As glands within cow are not biologically independent, the degree of correlation of results between glands needs to be examined (Barkema et al., 1997). Where the correlation is high, failure to account for the correlated nature of the data may lead to underestimation of the variance (Schukken, 2003), overestimation of the significance of results and the drawing of incorrect inferences. Dohoo et al., (2003, pg 459-68) states that ignoring clustering can lead to deficiencies other than variance inflation and even when no correlation or dependence is seen, cluster-adjusted methods should be used where the data are not independent. The intraclass correlation (ICC) of gland within cow was <0.18 for all variables, calculated using one-way analysis of variance for each dependent variable. An ICC of <0.2 suggests a low degree of correlation and hence that clustering is of limited importance. The variance inflation factor was calculated by accounting for the numbers of glands within cow and was also found to be small (i.e. <20%). To further examine the potential effects of non-independence of gland within heifer, a generalised linear model (GLIMMIX; SAS v 9.1, 2004) using the factors significant in the logistic regression models was developed, with and without adjusting for clustering by using a repeated measures statement for heifer and using a compound symmetry adjustment to account for correlation of gland within heifer. Compound symmetry adjusts for correlation by assuming that the correlation between glands within heifer is the same for every heifer. The –2log likelihood (model deviance) and error terms were compared for the two models for every final model and in each case the model deviance was not significantly different between the two models (P>0.1) and the standard errors were no larger in the models that adjusted for correlation. Therefore, as the correlation coefficients were small, and adjustment for correlation did not improve or alter the models, the outcomes from the simple logistic models were used for all dependent variables. Statistical significance was taken for test $P \leq 0.05$, and confidence intervals reported are for a 95% range of values. Data was recorded in a Microsoft Access database, and statistical analysis carried out using SAS version 9.1.

Results

One heifer was culled for being non-pregnant before calving start date and a further heifer removed from analysis, as the post-calving samples were not collected within 4 days of calving. None of the teat end swabs (those glands with no secretion collected; n=4) cultured any bacteria, and this was recorded as a 'missed sample' and dropped from further analysis. There were 6 glands that were non-functional post-calving and therefore excluded from the analysis.

Objective 1
The gland prevalence of IMI pre-calving was 15.5% (Table 5) and did not differ between herds (11.8-20.3%; P=0.69). The most common isolates were coagulase-negative staphylococcus (11.9% of glands) and *Streptococcus uberis* (2.2% of glands; Table 5).

Objective 2
The post-calving IMI mean gland prevalence for the control group was 12.3%, which did differ between herds (3.2-15.6%; P=0.001; Table 6). Coagulase-negative staphylococcus and *Streptococcus uberis* were the most common isolates (4.9% and 4.2% respectively; Table 6).

Presence of a pre-calving IMI, relative to no pre-calving IMI, increased the risk of post-calving IMI with any bacterial species (31/383 (8.1%) vs. 23/75 (30.7%), RR=3.58, (95% CI 2.03-5.55), P<0.001), with major bacterial pathogens (16/383 (4.1%) vs. 11/75 (14.7%), RR=3.32, (95% CI 1.40-6.36), P=0.001) and with *Streptococcus uberis* (9/383 (2.3%) vs. 6/75 (8.0%), RR=2.99, (95% CI 1.00-7.95), P=0.001; Table 8).

More specifically pre-calving IMI with coagulase-negative staphylococcus increased the risk of post-calving IMI with coagulase-negative staphylococcus, *Streptococcus uberis* and *Staphylococcus aureus* (Table 9).

The mean heifer and gland cumulative incidence of clinical mastitis for the control group were 21.4% and 6.7% respectively. The incidence differed across herds (P<0.001). More rear glands were diagnosed with clinical mastitis than fore glands (37/470 (7.9%) vs. 18/491 (3.7%), P <0.01).

Presence of a pre-calving IMI relative to no pre-calving IMI increased the risk of clinical mastitis with any bacterial species, (10/76 (13.2%) vs. 9/389 (2.3%), RR=4.78, (95%CI 1.85-11.1), P=0.001), and with clinical mastitis caused by only major pathogens (8/76 (10.5%) vs. 9/389 (2.3%), RR=3.76, (95% CI 1.41-9.37), P<0.001; Table 8). The prevalence of post-calving IMI declined with increasing number of days from calving to sampling post-calving (Figure 7).

Objective 3
Sampling pre-calving did not increase the risk of post-calving IMI (RR=1.05, (95% CI 0.75-1.48), P=0.77; Table 6) or the risk of clinical mastitis post-calving (RR =1.05, (95% CI 0.63-1.75), P=0.69; Table 7) relative to not sampling.

Objective 4
Forty of 440 (9.1%) glands acquired new infections between the pre and post-calving sampling. Of these, 13 were *Streptococcus uberis*, 16 were coagulase-negative staphylococcus, 6 were *Staphylococcus aureus* and 5 were other species. Sixteen (3.6%) glands had the same bacterial species isolated pre and post-calving and 54 (12.3%) glands apparently underwent spontaneous cure. Neither the presence of IMI pre-calving (9/76 (11.8%) vs. 27/380 (7.1%), RR=1.67, (95%CI 0.82-3.4), P=0.17), nor the use of a teat sealant (16/236 (6.8%) vs. 24/234 (10.3%), RR=0.69, (95%CI 0.38-1.3), P=0.23) significantly altered the risk of new infection.

Objective 5

Treatment with a teat sealant pre-calving tended to decrease the risk of post-calving IMI with any bacterial species (49/495 (9.9%) vs. 67/503 (13.3%), RR=0.74, (95%CI 0.52-1.06), P=0.10; Table 4) and with major bacterial pathogens (28/495 (5.7%) vs. 38/503 (7.6%), RR=0.73, (95%CI 0.46-1.21), P=0.1; Table 8), relative to no treatment with teat sealant. There was a significant decrease in the risk of post-calving IMI with *Streptococcus uberis* following the use of a teat sealant compared to no teat sealant (14/495 (2.8%) vs. 29/503 (5.8%), RR=0.47, (95%CI 0.26-0.92), P=0.02; Table 8). The RRs of post-calving IMI when pre-calving IMI status was adjusted for were 0.62, 0.43, and 0.16 for post-calving IMI with any bacterial species, post-calving IMI with a major pathogen and post-calving IMI with *Streptococcus uberis* respectively.

Objective 6

Teat sealant did decrease the risk of clinical mastitis from which any bacterial pathogen was isolated (30/501 (6.0%) vs. 10/501 (2.0%), RR=0.36, (95%CI 0.18-0.72), P=0.004) and clinical mastitis from which a major pathogen was isolated (24/501 (4.8%) vs. 7/501 (1.4%), RR=0.33, (95%CI 0.15-0.73), P=0.01; Table 8). The RRs of clinical mastitis when pre-calving IMI status was adjusted for were 0.36 and 0.33 for clinical mastitis that isolated any bacterial species, and clinical mastitis that isolated a major pathogen respectively. Teat sealant tended to decrease the risk of all clinical mastitis cases (34/501 (6.8%) vs. 21/501 (4.2%), RR=0.63, (95%CI 0.36-1.1), P=0.07). The number of clinical mastitis cases that had no bacterial pathogen cultured was higher in the glands treated with teat sealant (4/34 (11.8%) vs. 10/21 (47.6%), RR=4.2, (95%CI 1.5-11.6), P=0.003). Across all groups 69.1% of glands with clinical mastitis were bacteriologically positive (Table 7). The risk of clinical mastitis declined for heifers calving later in the season (P<0.07 for all models with a clinical mastitis outcome (Figure 8).

Discussion

Objective 1

This is the first time since 1970 (Munch-Peterson, 1970) that the prevalence of IMI has been estimated in pre-calving heifers in a pasture-based grazing system. The gland level prevalence of pre-calving IMI in the current study was 15.5%. This prevalence is lower than described in more intensive management systems where between 50% and 70% (Oliver et al., 1992; Oliver et al., 1997), and 97% of glands have been reported as infected (Trinidad et al., 1990a). The most prevalent pre-calving isolates in the current study were coagulase-negative staphylococcus (11.9%) in agreement with previous studies (Oliver and Mitchell, 1983; Fox et al., 1995; Aarestrup and Jensen, 1997).

Numerically the prevalence of *Streptococcus uberis* (2.2%) was similar, but was proportionally higher than reported in previous studies (Trinidad et al., 1990a; Fox et al., 1995). The prevalence of *Staphylococcus aureus* was lower in the current study (0.8%) compared to other studies where the *Staphylococcus aureus* prevalence was 9% to 15% (Trinidad et al., 1990a; Fox et al., 1995). Overall, the prevalence of major pathogens was lower in the current study (3%), than the 8 to 10% reported in previous studies (Oliver et al., 1992; Fox et al., 1995). This suggests that the prevalence of pre-calving IMI in heifers reared and calved under pasture-based management systems maybe lower than heifers reared and then calving under more intensive conditions.

Regional and seasonal variations in prevalence and relative frequency of IMI pathogens have been reported previously (Fox et al., 1995). This may be related to regional variation in managerial approaches, genetics of the animals or climatic conditions and hence bacterial survival (Fox et al., 1995). Specific regional factors such as presence of the horn fly, which has been shown to transmit *Staphylococcus aureus*, may be account for some regional variation (Fox et al., 1995; Owens et al., 2002). Higher proportions of cereals and lower proportions of rye grass in the diet are associated with higher bulk tank somatic cell counts (Barnouin et al., 1995) suggesting some association between diet and risk of IMI. Increasing production is also associated with increased risk of clinical mastitis (Chassagne et al., 1998; Waage et al., 1998). Thus the lower prevalence of IMI pre and post-calving, the lower incidence rate of clinical mastitis, and the different distribution of pathogens observed in the current study compared to previous studies in different production systems, may be related to a number of the above mentioned factors. The major differences in the production systems are that cows in New Zealand calve predominately in spring, that the majority of the cows' diet is pasture rather than concentrate feeds or silage, that the cattle are managed on pasture and not housed and that the production levels are generally lower than the more intensive production systems.

Objective 2

The post-calving IMI gland level prevalence of 12.3% in the control glands was lower than that reported in studies carried out in the USA (between 31% and 75%; Fox et al., 1995; Nickerson et al., 1995; Oliver et al., 2003). Clinical mastitis was diagnosed in 21.9% of heifers in the first 2 weeks after calving across all treatment groups in the current study, which was higher than the 8.1% diagnosed in the first 5 days after calving in a previous New Zealand study (Pankey et al., 1996). The higher incidence of clinical mastitis in the current study could be explained by climate/season or regional differences. In this study the five herds were not randomly selected, therefore the incidence of clinical mastitis may not reflect the New Zealand average incidence. As found in mature cows in New Zealand (Williamson et al., 1995; Pankey et al., 1996; McDougall, 1999), *Streptococcus uberis* was the most common pathogen isolated from clinical mastitis cases.

In the present study, IMI pre-calving was associated with an increased risk of IMI post-calving, in agreement with previous findings specifically for *Streptococcus dysgalactiae* (Aarestrup and Jensen, 1997). However, studies examining the presence of coagulase-negative staphylococcus IMI on the risk of subsequent new IMI have provided conflicting results. In the current study pre-calving IMI with coagulase-negative staphylococcus increased the risk of post-calving IMI with coagulase-negative staphylococcus, *Streptococcus uberis* and *Staphylococcus aureus,* despite the small number of IMIs with major pathogens. However, in this study no speciation was performed on the coagulase-negative staphylococcus isolates, therefore the post-calving isolated maybe the same infection or a new infection of coagulase-negative staphylococcus. This is contradictory to findings of studies in multiparous cows, which suggest that glands previously infected with minor pathogens, in particular coagulase-negative staphylococcus, are more resistant to subsequent infection than uninfected glands (Edwards and Jones, 1966; Rainard and Poutrel, 1988; Matthews et al., 1991). Glands infected with coagulase-negative staphylococcus showed a reduced rate of IMI following experimental challenge with *Staphylococcus aureus* compared to bacteriologically negative glands (Pankey et al., 1985). This has recently been supported recently by *in vitro* work, which showed that *Staphylococcus chromogenes* taken from the teat apex of heifers consistently inhibited all the growth of

Staphylococcus aureus, *Streptococcus dysgalactiae*, and *Streptococcus uberis* strains (De Vliegher et al., 2004b). It has also been demonstrated that glands infected with *Corynebacterium bovis* are less likely to become infected with major pathogens (Black et al., 1972; Rainard and Poutrel, 1988; Lam et al., 1997). Others, however, have demonstrated that glands with *Corynebacterium bovis* are significantly more likely to become infected with environmental *Streptococcus spp.* (Hogan et al., 1988) and *Streptococcus agalactiae* (Pankey et al., 1985). Green et al., (2002) attempted to investigate the conflicting evidence supporting the protective effects of *Corynebacterium spp.* and found that glands from which *Corynebacterium spp.* were isolated at drying off were at an increased risk of clinical mastitis, whereas the presence of *Corynebacterium spp.* in the late dry and post-calving samples were associated with a reduction in the risk of IMI and clinical mastitis. A possible explanation for this is that the protective effects of *Corynebacterium spp* (identified in many studies) are masked because glands with an IMI may be innately susceptible to repeat infection, irrespective of how protective the IMI with *Corynebacterium* spp. is. Understanding the long-term effects of the presence of minor pathogens in the gland requires further work, especially in primiparous cows. Based on the results from this study, strategies intending to reduce post-calving IMI and clinical mastitis in heifers, should aim to reduce the prevalence of IMI pre-calving, as well as minimising new infections during the pre-calving period.

Objective 3

Sampling glands pre-calving did not increase the risk of subclinical or clinical mastitis post-calving, in the current study despite the potential risk that removing the teat plug might increase the risk that bacteria may enter the teat canal and establish an IMI. Removing keratin from the teat canal pre-milking affects the ability of the teat to form a teat plug (Capuco et al., 1992), and consequently increases the risk of IMI (Dingwell et al., 2004). However in the current study, this was not found to be the case, which is in agreement with a previous study in multiparous cows (Green et al., 2002). The prevalence, function and composition of teat plugs in heifers pre-calving are not well defined and require further research.

Objective 4, 5 & 6

Insertion of teat sealant prior to calving reduced the risk of any IMI or an IMI with *Streptococcus uberis* post-calving between 53% and 84% and reduced the risk of a gland developing clinical mastitis caused by any bacteria within 2 weeks of calving by between 64% and 69%. There was no interaction between the infusion of the teat sealant and pre-calving IMI status, however the RR of effect of the teat sealant appeared different in the models (1-5 vs. 6-10). The presence of IMI increased the risk of post-calving IMI and clinical mastitis, which may have altered the apparent effect. The range of the effect of teat sealant on IMI for the different outcomes in different models are probably associated with the small numbers of glands with infections, as the CI overlap indicating that the numbers are statistically no different.

Teat sealants, when infused at the end of lactation in mature cows, reduce the incidence rate of new IMI over the non-lactation period (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). The teat sealant is likely to remain effective until it is physically stripped or sucked from the gland (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). Although numerically the teat sealant reduced the number of glands acquiring new infections pre-calving, this was not statistically significant. However, the small numbers of new infections precluded definitive rejection of the null hypothesis. Power analysis (PASS, 2001) suggested

that 474 glands per treatment group would be required to demonstrate a significant difference assuming a 50% decrease in new infection rate when the new infection rate was 10% in the control glands, with $\alpha<0.05$, and with a power of 80% ($\beta=0.8$). Additionally, a new IMI due to the same species would not have been detected in the current study as no genotyping of bacterial isolates was undertaken. Thus use of genotypic techniques may also have improved the power of the study to detect new IMI.

The higher occurrence of clinical mastitis cases from which no bacteria were isolated in the glands treated with the teat sealant has not been reported previously. We hypothesise that the increased proportion of 'no growths' was associated with an increased proportion of false positives of clinical mastitis by the herd-owners based on the presence of white flecks of teat sealant in the milk. Huxley et al., (2002) reported flecks present in the milk for up to 3 weeks post calving in some cows following treatment with the teat sealant at the end of the lactating period. Poor letdown of milk by heifers during the first few milkings may increase the duration over which flecks are detected in the milk. Previous studies demonstrating the efficacy of a teat sealant have been in cows, which do not have as high as an incidence of clinical mastitis cases so close to calving as do heifers (Barkema et al., 1998), which may explain why this higher proportion of 'no growths' has not been reported previously.

Other findings

The decline in prevalence of post-calving IMI with increasing days after calving at sampling was expected. The physical removal of bacteria and somatic cells from the gland with twice daily milking after calving rapidly decreases the number of cells present in milk (De Vliegher et al., 2004a). Somatic cell count and Californian Milk Test scores decreased linearly in both infected and uninfected glands from the day of calving until 10 days post-calving (Sargeant et al., 2001), suggesting that bacteria and somatic cells are being cleared from the gland.

The decline in percentage of glands with clinical mastitis relative to the median calving date of the herd was an interesting finding. In the seasonal calving system 50-70% of heifers calve in the first 3 weeks of the calving period. This study suggests that at the peak of the heifer calving period there is a greater percentage of clinical mastitis. This may be climate related, where wet and cold conditions have occurred before the median calving date, with resultant increased faecal contamination on the udder and an increased risk of IMI (Schreiner and Ruegg, 2003). This finding could also be a result of time pressures on labour. For example, due to a large number of heifers (and cows) calving, the attention to detail by farm staff may have been compromised, so that calves may have been left with their dams for longer or heifers left on faecally contaminated pasture for longer. This finding needs to be examined further in multiple herds over multiple seasons to clarify its importance.

In the current study, the calculated intraclass correlation between glands was only moderate (i.e. ICC<0.2) and the use of models that accounted for the clustering of gland within cow did not result in changes in estimates of variance or to inferences compared to simpler models that did not account for clustering. The risk of *Staphylococcus aureus* being isolated from a gland from a lactating cow is increased by the presence *Staphylococcus aureus* in another gland within the same cow, illustrating that glands are not independent within a cow (Zadoks et al., 2001). This discrepancy between studies may be related to the fact that the most common 'major'

pathogen isolated in this study was *Streptococcus uberis*, which maybe defined as an 'environmental' pathogen (Todhunter et al., 1995). Thus, although evidence does exist of potential cow to cow transmission (Zadoks et al., 2003), it is likely that that major source of this pathogen is external to the cow. Additionally, heifers in the current study were sampled before and within 4 days of calving, so that exposure to the milking process and hence the risk of cross infection within the heifer via the milking claw may be relatively low.

Conclusions

The prevalence of pre-calving IMI in heifers in New Zealand is lower than in previous studies undertaken in the Northern Hemisphere. Pre-calving IMI is a significant risk factor for IMI post-calving and clinical mastitis in early lactation. Sampling of the glands pre-calving did not increase the risk of infection post-calving. Insertion of a teat sealant approximately 30 days before calving decreases the risk of post-calving IMI with *Streptococcus uberis* and clinical mastitis in the first 14 days of lactation. The findings of the current study present farmers with an alternative and practical treatment for reducing the prevalence of IMI and clinical mastitis in heifers post-calving.

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Table 5 Count (n) and percentage (%) of glands with intramammary infection (IMI) on average 31 days before the start of a seasonal calving period pre-calving for glands, which were sampled with no further treatment (S) or sampled and then infused with a teat sealant (TS+S).

	S		TS+S		Total	
Bacterial species cultured	n	%	n	%	n	%
No growth	185	72.8	202	81.1	387	76.9
Coagulase-negative staphylococcus spp.	34	13.4	26	10.4	60	11.9
Corynebacterium spp.	2	0.8	1	0.4	3	0.6
Staphylococcus aureus	4	1.6	0	0.0	4	0.8
Streptococcus uberis	9	3.5	2	0.8	11	2.2
Contaminated sample[2]	20	7.9	18	7.2	38	7.6
Total pre-calving IMI[1]	49	19.3	29	11.6	78	15.5
Total glands	254	100	249	100	503	100

[1] Intramammary infection
[2] >3 distinct colony morphology types present

Table 6 Count (n) and percentage (%) of glands with intramammary infection (IMI) within 4 days after calving for glands within heifers not sampled or treated (C), infused with a teat sealant (TS) pre-calving, sampled only pre-calving (S) or sampled and infused with a teat sealant pre-calving (S+TS).

Treatment of Gland	C		TS		S		S + TS		Total	
	n	%	n	%	n	%	n	%	n	%
No growth	221	87.7	224	88.2	217	85.8	224	90.7	886	88.1
Coagulase-negative staphylococcus	13	5.2	10	3.9	15	5.9	11	4.5	49	4.9
Staphylococcus aureus	4	1.6	5	2.0	4	1.6	4	1.6	17	1.7
Streptococcus uberis	14	5.6	11	4.3	14	5.5	3	1.2	42	4.2
Streptococcus dysgalactiae	0	0	1	0.4	1	0.4	0	0	2	0.2
Escherichia coli	0	0	2	0.8	1	0.4	1	0.4	4	0.4
Pseudomonas spp.	0	0	0	0	0	0	1	0.4	1	0.1
Yeast	0	0	0	0	1	0.4	0	0	1	0.1
Contaminated sample [2]	0	0	1	0.4	0	0	3	1.2	4	0.4
Total IMI [1]	31	12.3	29	11.4	35	13.8	20	8.1	115	11.4
Total glands	252	100	254	100	253	100	247	100	1006	100

[1] Intramammary infection
[2] >3 distinct colony morphology types present

Table 7 Count (n) and percentage (%) of bacteriological glands within all clinical mastitis glands from heifers diagnosed from 3 days before to 14 days post-calving, from glands treated approximately 31 days before the start of a seasonal calving programme with nothing (C), infused with a teat sealant (TS) pre-calving, sampled only (S) or sampled and infused with a teat sealant (S+TS).

Treatment of Gland	C		TS		S		S + TS		Total	
	n	%	n	%	n	%	n	%	n	%
No growth	3	17.6	3	33.3	1	5.9	7	58.3	14	25.5
Coagulase-negative staphylococcus	4	23.5	1	11.1	2	11.8	0	0	7	12.7
Staphylococcus aureus	1	5.9	0	0.0	1	5.9	1	8.3	3	5.5
Streptococcus uberis	9	52.9	3	33.3	13	76.5	2	16.7	27	49.1
Escherichia coli	0	0	0	0	0	0	1	8.3	1	1.8
Fungi	0	0	2	22.2	0	0	0	0	2	3.6
Contaminated sample	0	0	0	0.0	0	0	1	8.3	1	1.8
Total glands pathogen positive	14	82.4	4	44.4	16	94.1	4	33.3	38	69.1
Total clinical mastitis of all glands	17	100	9	100	17	100	12	100	55	100
Total number of glands with clinical mastitis	17	6.7	9	3.5	17	6.8	12	4.9	55	5.5
Total glands	252		254		249		247		1002	

Table 8 Relative risks (RR) from the multivariate analyses and 95% confidence intervals (CI) with probability values (P) calculated from the final logistic regression models for the dependent variables. Models 1-5 include pre-calving bacteriology status (n=503) as a variable in the model, models 6-10 do not (n=1006). Herd is included in all models as a fixed effect. Days from calving to sampling were included as a categorical variable in all post-calving IMI models (models 1-3, 6-8) and days calved relative to the group median calving date in the clinical mastitis models (models 4-5, 9-10).

	Pre-calving IMI not adjusted for[3]					Pre-calving IMI adjusted for[3]				
			CI					CI		
Dependent variables	Model	RR[2]	Lower	Upper	P	Model	RR[2]	Lower	Upper	P
Post-calving IMI[1]	1	0.62	0.35	1.07	0.08	6	0.74	0.52	1.06	0.10
Post-calving *Streptococcus uberis* IMI[1]	2	0.16	0.04	0.73	0.02	7	0.47	0.26	0.92	0.02
Post-calving IMI[1] with a major pathogen	3	0.43	0.20	1.03	0.06	8	0.73	0.46	1.21	0.10
Clinical mastitis culturing any pathogen	4	0.32	0.10	0.96	0.04	9	0.36	0.18	0.72	0.00
Clinical mastitis culturing a major pathogen	5	0.36	0.11	1.10	0.07	10	0.33	0.15	0.73	0.01

[1] Intramammary infection
[2] Reference group for RR in all models is no treatment with teat sealant pre-calving
[3] There was no interaction between pre-calving IMI and treatment in any of the models

Table 9 Crude relative risks (RR) with 95% confidence intervals (CI) and probability values (P) for the outcome post-calving intramammary infection (IMI), in the presence or absence of a pre-calving IMI with coagulase-negative staphylococcus (cns).

	Pre-calving IMI			95% CI		
Post-calving IMI	cns	No growth	RR	Lower	Upper	P
No growth	40	352	0.76	0.6	0.9	<0.001
cns	10	12	5.57	2.5	12.3	<0.001
Staphylococcus aureus	3	3	6.68	1.4	32.3	0.007
Streptococcus uberis	4	8	3.34	1	10.7	0.03
Other[1]	0	6				
Total	57	381				

[1] *Streptococcus dysgalactiae, Escherichia coli, Pseudomonas* spp. and Yeast

Figure 6 The four treatment allocations randomly assigned to groups of four sequentially presented heifers within a herd. Each large box represents the four glands within a heifer with the smaller boxes one of the four glands, clockwise from top left; left front, right front, right rear, left rear. TS= teat sealant infused, S=sample taken, S+ TS = sample taken and teat sealant infused, C=control

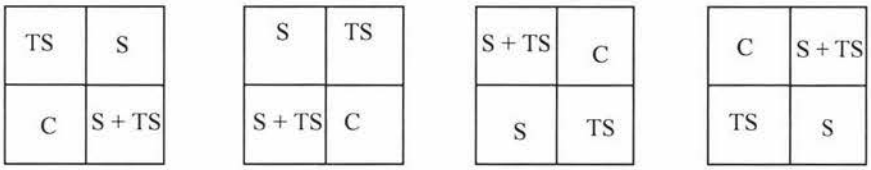


Figure 7 Mean (± SEM) prevalence as a percentage of glands with intramammary infection (IMI) by day post-calving at milk sampling.

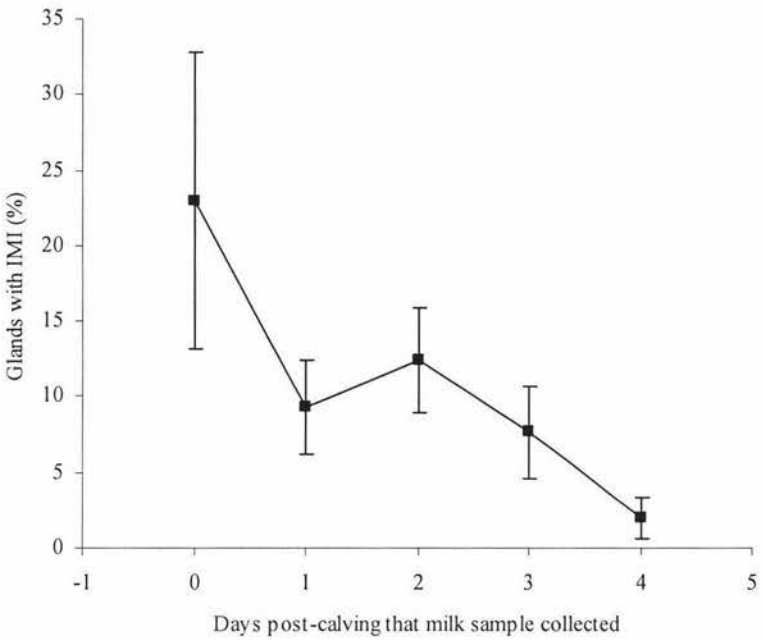
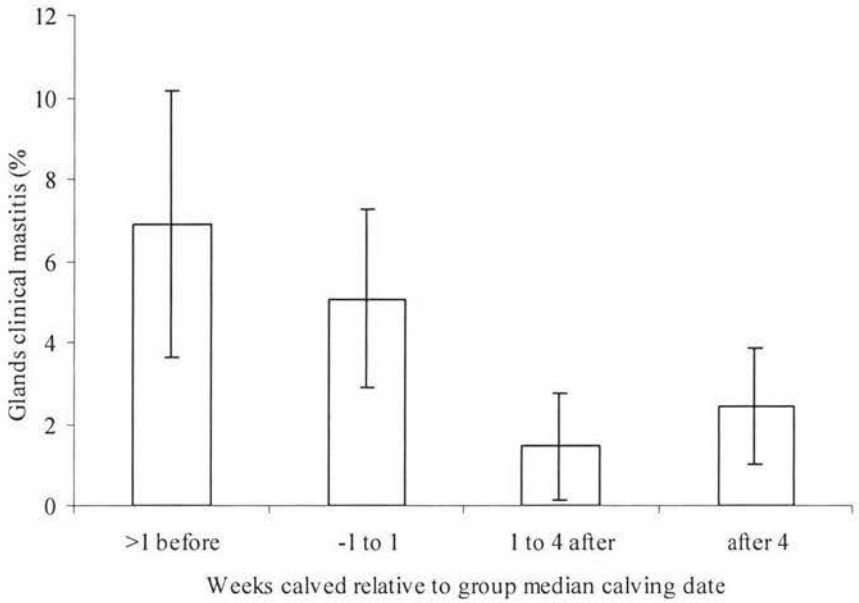


Figure 8 The cumulative incidence of clinical mastitis (±SEM) within herd from which any pathogen was isolated by week of calving relative to the median calving week for heifers within a herd.



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Chapter 5 Quarter level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant and/or an injectable antibiotic pre-calving

Abstract

This study investigated the effect of infusion of a bismuth subnitrate teat canal sealant and/or and injectable antibiotic on the incidence of cure of intramammary infection (IMI), new infection rate of IMI, prevalence of post-calving IMI and incidence of clinical mastitis in the first two weeks post-calving at quarter-level in heifers. Glands (n=4268) from heifers (n=1067) in 30 seasonally calving, pasture-fed dairy herds were randomly assigned at heifer level to one of four treatment groups (no treatment; three intramuscular injections of 5g of tylosin antibiotic; infusion of a teat sealant into all four quarters; three intramuscular injections of 5g of tylosin antibiotic and infusion of teat sealant into all four quarters). Mammary gland secretion samples were collected from each quarter of every heifer before treatment. Heifers within a herd were enrolled on one calendar day, on average 39 days before the planned start of the seasonal calving period. Duplicate milk samples were collected from each gland within five days after calving for bacterial culture, and from glands the herd-owners diagnosed as having clinical mastitis. The relative risk of the incidence of cure, incidence of new IMI, post-calving infection and clinical mastitis were calculated using Mantel Haenszel and logistic regression analyses. Neither infusion of a teat sealant nor treatment with the injectable antibiotic increased the risk of cure of pre-calving IMI. Infusion of the teat sealant reduced the risk of new IMI with any pathogen by 74%, reduced the prevalence of post-calving IMI by 65%, reduced the risk of new infection with *Streptococcus uberis* by 73%, and reduced the incidence of clinical mastitis from which a pathogen was isolated by 74%. In conclusion use of an internal teat canal sealant in heifers did not have an effect on cure of existing IMI but reduced the risk of post-calving IMI and clinical mastitis post-calving by decreasing the incidence of new infections over this high-risk peripartum period, and provides a useful tool for reducing the risk of subclinical and clinical mastitis in heifers.

(***Keywords***: mastitis, *Streptococcus uberis*, heifer, teat sealant, antibiotics)

***Abbreviation key*: IMI** = intramammary infection, **ICC** = Intraclass correlation, **RR** = Relative risk, **OR** = Odds ratio

Introduction

Studies in several countries have shown high incidence of clinical mastitis and intramammary infection (IMI) in first-calving heifers peripartum relative to multiparous cows (Pankey et al., 1991; Barkema et al., 1998b; Barnouin and Chassagne, 2001). Pankey et al., (1996) reported that an average 8.1% of heifers in eleven pasture-grazed New Zealand dairy herds were diagnosed with clinical mastitis within 5 days of calving, and that the environmental pathogen *Streptococcus uberis* was isolated from 67.6% of these cases.

Bacteria have been isolated from the mammary gland of heifers as early as 9 months pre-calving (Trinidad et al., 1990a) and the prevalence of infection increases as calving approaches (Oliver and Mitchell, 1983; Aarestrup and Jensen, 1997), with prevalence's reported to range from 20% to 97% in heifers pre-calving (Meaney, 1981; Trinidad et al., 1990a; Myllys, 1995). Parker et al, (unpublished, chapter 4), found the gland-level prevalence of IMI ~ 30 days pre-calving in primiparous heifers in New Zealand to be 15.5%. There is an increased risk of new IMI and clinical mastitis peripartum relative to the earlier gestational period, associated with the transition into lactation (Aarestrup and Jensen, 1997).

The relative prevalence of specific pathogens changes across the peripartum period (Oliver and Mitchell, 1983; Aarestrup and Jensen, 1997). Glands may change IMI status by acquiring a new IMI or undergoing spontaneous cure of an existing IMI pre-calving (Aarestrup and Jensen, 1997). The effect of the presence of an IMI pre-calving on the risk of a new IMI is still unclear. Some studies in multiparous animals have shown that existing IMI with a minor pathogen may reduce the prevalence of IMI with major pathogens at calving and in early lactation (Rainard and Poutrel, 1988; Lam et al., 1997a; Green et al., 2002). However, other studies have found that an existing IMI with a minor pathogen increases the risk of infection with another pathogen (Pankey et al., 1985; Hogan et al., 1988).

Specific prevention programmes for mastitis in dairy heifers are not currently available. Current mastitis prevention is targeted at multiparous animals, which primarily focuses on prevention of spread and elimination of the contagious pathogens (Smith et al., 1967; Woolford et al., 1995). Clinical mastitis in heifers occurs primarily early in lactation (Barkema et al., 1998a; Compton et al, unpublished) and in pasture-grazing systems is commonly caused by the environmental pathogen *Streptococcus uberis* (Pankey et al., 1996; McDougall, 1999; Compton et al, unpublished), hence programmes which are effective at reducing mastitis in cows in lactation, may not be effective for heifers in the periparturient period.

Infusion of intramammary antibiotics pre-calving reduces the prevalence of IMI and the incidence of clinical mastitis post-calving in heifers (Oliver et al., 1992; Oliver et al., 1997; Oliver et al., 2003). However, use of antibiotics may increase the risk of residues in milk, and new infections may still be acquired before calving, as the period over which the antibiotics remain above the minimum inhibitory concentration in the glands is highly variable between heifers (Trinidad et al., 1990b; Oliver et al., 1992). In seasonally calving dairy herds, intramammary administration of antibiotics to every gland of every heifer presents logistical problems due to the large numbers of animals calving in a short period and therefore needing to be treated. Parenteral antibiotic therapy has been successfully used in treating clinical mastitis in lactating cows (Ziv and Storper, 1985; McDougall, 1998; St Rose et al., 2003), and may be

more practical than intramammary therapy. Tylosin (Tylan 200, Elanco Animal Health, Manukau City, New Zealand) has been used to treat clinical mastitis in dairy cows, based on the pH of the compound allowing transfer into the mammary gland tissues (McDougall et al, unpublished).

The infusion of a teat sealant into the teat canal at the end of lactation has been shown to reduce the incidence of new IMI during the dry period of mulitparous cows (Meaney, 1977; Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). In a pilot study, infusion of a teat sealant approximately 4-6 weeks before calving was found to be effective at decreasing the prevalence of post-calving (0-4 days) IMI of *Streptococcus uberis* in heifers by 84% (Parker et al., unpublished, chapter 4). This study had limited statistical power and a larger field study was required to confirm the preliminary findings.

Therefore the hypotheses of this study were that the infusion of a teat sealant and/or treatment with tylosin in heifers approximately 30 days before the planned start of calving from seasonal-calving dairy herds, would result in:

1. An increased incidence of cure of existing IMIs between the pre-calving treatment and 0 to 5 days post-calving,
2. A reduction in the number of new quarter level IMI's of any bacteria between pre-calving treatment and 0 to 5 days post-calving,
3. A reduction in the number of new quarters infected with *Streptococcus uberis* between the pre-calving treatment and 0 to 5 days post-calving,
4. A reduction in the prevalence of quarter level IMI between day 0 and 5 post-calving, and,
5. A reduction in the incidence of quarter level clinical mastitis up to 14 days post-calving.

Materials and methods

Glands (n=4268) from heifers (n=1067) in 30 spring calving, pasture-fed dairy herds were enrolled in a field study. The herds were located in the Waikato region of the North Island of New Zealand. Between 18 and 48 heifers were enrolled from each herd. The herds ranged in size from 214 to 810 cows (median=310). Herds were selected on the basis that herd-owners would undertake 4 herd tests for milk production and composition in the season of the study, used an electronic database (Livestock Improvement Corporation, Hamilton) to record animal details, farmed within a radius of 30 km of one veterinary practice, maintained high quality cow records and agreed to comply with the trial protocol.

A priori a sample size of 1000 heifers was calculated based on the assumption that the gland level prevalence of IMI post-calving would be decreased by the teat sealant and antibiotic treatment using an absolute effect size of 0.05 (W), which is a measure of the magnitude of the Chi-square of the difference, (Hintze, 2001, NCSS and PASS). A preliminary study estimated gland level prevalence of IMI to be ~ 16% pre-calving and 12% post calving. Teat sealant reduced the prevalence of post-calving IMI from ~ 12.1% to 9.7% and cumulative incidence of clinical mastitis from ~ 6.9% to 4.5% (Parker et al unpublished, chapter 4). Hence, to demonstrate a 5% difference (with a 95% confidence limit (α=0.05) and 80% power (β=0.2)) in clinical mastitis incidence, with the control group having an incidence of 10%, then 474 glands/group were required.

Heifers in each herd were enrolled on one calendar day, on average 18 to 37 days before the start of the seasonal calving period (median=27). The heifers calved between 5 and 127 days after enrolment (median= 39). Treatment was assigned within herd in a 2 by 2 factorial arrangement at heifer level. Each heifer was randomly assigned to one of five treatment groups using a random number generator function in "R: A language and environment for statistical computing" (R Development Core Team, 2005) version 2.2.0. The number of heifers available in each herd and the number required for this study determined the sample size from each herd. Heifers were assigned to two control groups, however one of the control groups was used only in an observational study to be reported elsewhere (Compton et al., unpublished).

Before treatment, each teat end was scrubbed with a cotton wool pledget moistened in 70% methanol and a gland secretion sample collected aseptically. No secretion was discarded before collection due to the small volume of secretion present in most glands. Where no secretion could be collected from a gland it was recorded as a missing sample (n=99 glands). Following sampling, all four glands within a heifer were either infused with 2.6g of bismuth subnitrate following teat end scrubbing (n=268; Teat Seal, Pfizer Animal Health NZ Ltd, Auckland, New Zealand), or a heifer was administered with 5 g intramuscularly of tylosin for 3 days at 24 hourly intervals (n=268; Tylan 200, Elanco Animal Health, Manukau City, New Zealand), or all four glands were infused with the teat sealant and the heifer was administered 5 g of tylosin i.m. daily for 3 days (n=266), or they were left as an untreated control (n=265). The tip of the teat sealant cannula was inserted approximately 3 mm into the teat canal for infusion. Following sampling or infusion, 0.5% effective iodine was applied by manual spraying to all teat ends. Technicians administered the first treatment of tylosin and then left labelled, 'pre-drawn' doses of tylosin for the remaining two treatments on farm, for the farm staff to administer at subsequent 24 hourly intervals.

The body condition score on a 1-10 scale in half score increments (Roche et al., 2004), the udder and escutcheon hygiene score on a 1-4 scale (Schreiner and Ruegg, 2003), the length of the tail of each heifer (ordinal scale: 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) and presence of udder oedema (as determined by digital pressure in the caudal aspect of the udder resulting in the presence of a depression for >30 secs) were recorded for each animal at enrolment and again at the post-calving sampling, except teat height which was only recorded at the post-calving sampling.

Within 5 days of calving (range 0 to 5 days after calving; median=2 days), duplicate milk samples (~ 20 ml each) were collected from each gland, following aseptic preparation of the teat end and discard of the first 3 strippings. Herd-owners were asked to diagnose clinical mastitis based on the presence of visible changes (e.g. clots or blood) in the milk composition or swelling and/or pain in gland. Duplicate milk samples (~ 20 ml each) were collected from each gland by technicians from glands with which were diagnosed with clinical mastitis within 14 days of calving, following aseptic preparation of the teat end and discard of the first 3 strippings. Where clinical mastitis was diagnosed on or before the scheduled day 0-5 post-calving visit, all glands as well as the gland with clinical mastitis were sampled and the bacteriology results were included in both the post-calving 0-5 day analysis (post-calving IMI) and

the clinical mastitis analysis. Calving date and breed data were retrieved from the national database (Livestock Improvement Corporation, Hamilton, New Zealand).

Bacteriology

Pre-calving secretion samples, post-calving milk samples and clinical mastitis samples were processed for bacteriology within 24 hours of collection following storage at 4°C.

Microbiological procedures, diagnosis of IMI, and categorisation of results were undertaken using standard methodology with minor modifications (National Mastitis Council, 1999, USA). Following gentle inversion at room temperature, 10 µl of milk was streaked onto a quadrant of a 5% sheep blood agar plate containing 0.1% esculin (Fort Richard, Auckland, New Zealand), and incubated at 37°C for 48 h. For post-calving, which were collected in duplicate, both samples were plated onto a quadrant of the same plate for easy comparison of results. The genus of bacteria was provisionally determined on the basis of colony morphology, Gram stain, haemolysis pattern, catalase test and esculin reaction. Gram-positive, catalase-positive isolates were further tested using a tube-coagulase test and coagulase-positive isolates were defined as *Staphylococcus aureus* and coagulase-negative isolates as coagulase-negative staphylococcus. Gram-positive, catalase-negative isolates were CAMP tested and esculin-positive and CAMP-positive or negative isolates were defined as *Streptococcus uberis*, esculin-negative and CAMP-negative isolates as *Streptococcus dysgalactiae* and esculin-negative and CAMP-positive isolates as *Streptococcus agalactiae*. Gram-negative rods were sub-cultured on MacConkeys agar, had an oxidase test performed and were cultured in triple sugar iron agar and simmons citrate agar. Gram-negative rods that could be identified were 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.

A milk sample was defined as contaminated if >2 distinct colony types were found present. A gland was defined as infected where 3 or more of each of 1 to 2 colony morphology types were present. *Bacillus* spp., Fungi or <3 colonies present were defined as "no growths".

Data Handling

Results from samples and measurements that were collected >5 days post-calving that were collected for routine sampling (n=13 heifers, 52 glands) or for the clinical mastitis data for cases of clinical mastitis that occurred >14 days after calving were discarded from the analysis. Twelve heifers did not calve (n=48 glands) and one heifer was not sampled post-calving (n=4 glands). The data analysis was conducted on 1041 heifers and 4164 glands.

Contaminated samples were not included in the denominator when proportions were calculated. A new infection was defined as occurring where a bacterial species was isolated from a gland post-calving that had not been isolated pre-calving. Cure was defined as occurring where a gland from which a bacterial species was isolated pre-calving either did not isolate any bacteria or isolated a different bacterial species from the pre-calving species. A gland may therefore be positive for both a cure and a new infection.

The gland level outcomes were analysed only for the first bacterial species isolated. Where there were secondary species they were recorded in counts, in brackets next to the primary species (Table 10-12). Any major pathogen present was assigned as the IMI of interest (primary). Where two minor pathogens were present coagulase-negative staphylococcus was assigned as the IMI of interest (primary). There were 10 quarters across all milk samples where 2 major pathogens were isolated. Eight of these samples isolated *Escherichia coli*, which was assigned as the secondary pathogen. For two remaining samples *Staphylococcus aureus* was assigned the primary pathogen.

Data analysis

Descriptive statistics at gland level were initially performed. Continuous variables that were normally distributed remained as continuous variables in subsequent analysis. However, if the relationship between this continuous variable and the outcome variable was not linear the variable was categorised or transformed. Body condition score post-calving was categorised into 5 groups (<4.5, 4.5, 5, 5.5, >5.5). Breed of heifer was defined as the predominant parentage breed if greater than $11/16^{th}$ (Friesian or Jersey), while all remaining animals were classified as crossbreds.

Dependent variables (all binary) included cure of IMI, the presence of a post-calving IMI, clinical mastitis, clinical mastitis from which any pathogen was cultured, new infection with any pathogen, and new infection with *Streptococcus uberis*. A high prevalence of clinical mastitis milk samples from which no pathogens were isolated was found in a pilot study (47.6% in glands treated with teat sealant; Parker et al., unpublished, chapter 4). This may have occurred due to the false diagnosis of clinical mastitis by the herd-owners, following detection of teat sealant in the milk. To account for this, analyses of all clinical mastitis cases as well as clinical mastitis from which bacteria were isolated, were undertaken. Clinical mastitis (including those cases from which no pathogen was isolated) was only analysed at the bivariate level, as the multivariate models were unstable.

Fixed effects that were examined in the final models were the categorical variables: infusion of a teat sealant, treatment with tylosin, breed, pre-calving IMI (present/absent), presence of udder oedema at pre and post-calving (binomial; present or absent) and body condition score at 0-5 days post-calving, and the continuous variables; interval between infusion and calving (days), number of days post-calving at sample collection (0-5 days), interval from treatment to calving (days) and the minimum height (cm) of the teat end from the ground.

Bivariate associations between independent and dependent variables were examined using chi-square analysis and the crude relative risks (RR) were calculated. The Mantel-Haenszel procedure was used to stratify the data by some of the covariates such as pre-calving IMI and the adjusted RRs were calculated. If the treatment did not significantly affect the outcome variable at bivariate level, further modelling was not performed and the bivariate RRs were reported. Predictor variables from the bivariate and stratified analyses associated with the outcome variables (i.e. $P<0.2$) were manually added in a step-wise manner to the generalised mixed log binomial model (GLIMMIX, SAS v 9.1, 2004). The variables were added to the model and tested in descending order of significance at bivariate level.

Variables were included in the mixed models if they reached significance ($P<0.05$) or were integral to the study design (i.e. the main treatment effects). Biologically meaningful interactions were tested including teat sealant by tylosin, teat sealant by pre-calving IMI status, teat sealant by calving to sampling time and teat sealant by post-calving udder oedema. If the interaction was not significant and it did not alter the coefficient of the main effects by >10% then the interaction was not included in the final model. Some covariates remained in the model, despite the fact they were not confounders (did not alter the main effect coefficients by >10%), because they explained a large amount of variation in the model, improved the model fit (reduced the model deviance) and it made biological sense to include them.

The smallest –2log likelihood (model deviance), type 3 sums of squares for each variable added, and the coefficients and error terms of the main effects were used to determine the final model. Model diagnostics were performed, including plotting the residuals against the predicted values and investigating the outliers.

Where the prevalence of a disease outcome is >10% odds ratios (OR) overestimate the size of the effect and RRs are a better estimate of the effect size (Dohoo et al., 2003; McNutt et al., 2003). Logistic regression models which use a logit link function (in SAS software), produce coefficients which when exponentiated produce OR. However, exponentiation of coefficients from log-binomial models which use the log link function in SAS software produce an estimate of the adjusted RR (McNutt et al., 2003).

As glands within heifers are not biologically independent, and treatments were assigned at heifer level, the degree of correlation of results between glands within heifers, and between heifers within herds needed to be examined (McDermott et al., 1994; Barkema et al., 1997). The intraclass correlations (ICC) of gland within heifer and heifer within herd were calculated using one-way analysis of variance for each dependent variable and were significant (i.e. ICC>0.2). Therefore, in the final models, herd was included as a random effect statement to account for correlation within herds, and heifer nested within herd was included as a random effect statement to account for correlation within cow.

The only final model that was not a mixed model was that with the outcome variable being new infection with *Streptococcus uberis.* Inclusion of the random effect statements for herd and heifer nested within herd resulted in an unstable model outcome. This was because there were a large number of herds with no new infection of *Streptococcus uberis* in either the teat sealant or the non-teat sealant treated quarters (i.e. some cells were=0). A marginal binomial logistic regression model (PROC GENMOD) was used with herd included as a fixed effect and a compound symmetry repeated statement included for cow to account for correlation within cow. This model was not over dispersed (over dispersion = deviance/degrees of freedom >1) which supports the validity of this model (Zadoks et al., 2001). The coefficients of this logistic regression model were exponentiated to give the ORs. The quarter level incidence of new infection of *Streptococcus uberis* across all herds was 5.7%, hence OR are an acceptable approximation of RR in this case (McNutt et al., 2003). The effects were reported as RRs.

Statistical significance was taken for test $P \leq 0.05$, and confidence intervals (CI) reported are for a 95% range of values. Data were recorded in a Microsoft Access database, and statistical analysis carried out using SAS version 9.1.

Results

The gland level prevalence of pre-calving IMI was 16.8%, and differed between herds (7.4-27.3%; P<0.001). Coagulase-negative staphylococcus (75.5% of glands with IMI) was the most commonly isolated pathogen and *Streptococcus uberis* the next (15.6%; Table 10). The post-calving IMI gland prevalence for the control group was 21.2% (Table 11), which also differed between herds (4.0-30.7%; P<0.001). The distribution of bacterial species is shown in Table 11. The incidence of new infection with any bacterial species was 15.7% in the control group. The cumulative incidence of clinical mastitis in the control group in the period up to 14 days post-calving was 6.6% (Table 12). The most common species isolated from clinical mastitis cases was *Streptococcus uberis* (34.9% of all clinical mastitis cases).

There were four treatment groups to be assessed in the data analysis. However, the effect of tylosin was not significant at univariate level for any of the outcome variables tested and the interaction between teat sealant and tylosin was tested but was not significant in any of the models. Therefore the analysis was performed with 2 main treatment effects: infusion of the teat sealant (dichotomous) and treatment with tylosin (dichotomous).

The ICCs were calculated for each outcome variable within heifer and within herd. The post-calving IMI ICC for within heifer was 0.31 being far greater than the correlation within herd (ICC=0.08).

Effect of treatment on cure of existing IMI

Neither the infusion of a teat sealant (254/2084 (12.2%) vs. 257/2080 (12.4%), RR=0.99, P=0.9) nor the administration of tylosin (256/2100 (12.2%) vs. 255/2064 (12.4%), RR=0.99, P=0.87) increased the number of glands that cured between the pre-and post-calving sampling days.

Effect of treatment on incidence of new IMI

Teat sealant decreased the risk of new IMI with any bacteria (RR=0.34, (95%CI 0.26-0.43), P<0.001). Treatment with tylosin did not significantly decrease the risk of new infection (RR=1.08, (95%CI 0.84-1.38), P<0.001). There were no significant interactions present in this model (Table 14).

The incidence of new infection with *Streptococcus uberis* in the control group was 7.8%. Teat sealant decreased the risk of new infection with *Streptococcus uberis* (50/2080 (9.0%) vs. 187/2084 (2.4%), Crude RR=0.27, (95%CI 0.20-0.36), P<0.001). There was a significant interaction (P=0.049) between infusion of the teat sealant and pre-calving IMI status. There was a greater risk of post-calving IMI in quarters with pre-calving IMI in the absence of teat sealant (RR=2.0, (95%CI 1.38-2.89), P=0.003), therefore there appeared to be a greater reduction in new IMI of *Streptococcus uberis* following infusion of the teat sealant in glands that had an IMI pre-calving (RR=0.14, (95%CI 0.06-0.33), P<0.001) compared to glands that did not have an IMI pre-calving (RR=0.33, (95%CI 0.21-0.47), P<0.001). However, there was no difference in the

effect of teat sealant in the presence of absence of pre-calving IMI (RR=0.9, (95%CI 0.41-2.01), P=0.81). Tylosin did not decrease the risk of new infection with *Streptococcus uberis* (RR=1.23, (95%CI 0.88-1.7), P=0.22).

Effect of treatment on prevalence of IMI post-calving

The use of a teat sealant pre-calving decreased the risk of any IMI post-calving (154/2080 (7.4%) vs. 458/2084 (22.0%), RR=0.35, (95%CI 0.28-0.4), P<0.001; Table 13). Tylosin treatment did not alter the risk of IMI post-calving (RR=1.02, (95%CI 0.82-1.27), P=0.84). Presence of pre-calving IMI increased the risk of IMI post-calving (RR=3.25, (95%CI 2.82-3.74), P<0.001). There was no interaction present between presence of pre-calving IMI and the teat sealant (P=0.09). Jerseys were at decreased risk and Friesians at increased risk of IMI post-calving relative to crossbreds (Table 13). There was an inverse relationship between the time between calving and sampling and the risk of isolating an IMI (Table 13; Figure 9, P<0.001). Increasing body condition score was associated with an increased risk of IMI (Table 13; Figure 10, P<0.04). Increasing distance of the teat end from the ground was associated with a decreased risk of IMI (Table 13; Figure 11, P=0.01)

Effect of treatment on incidence of clinical mastitis

Teat sealant did not decrease the risk of all clinical mastitis cases (143/2084 (6.9%) vs. 164/2080 (7.9%), RR=1.2, (95%CI 0.93-1.4), P=0.20). However, teat sealant did decrease the risk of clinical mastitis from which any bacterial pathogen was isolated (34/2080 (1.6%) vs. 130/2084 (6.2%), Crude RR=0.26, (95%CI 0.18-0.38), P<0.001; Table 15). The risk of clinical mastitis was increased due to the presence of an IMI pre-calving and there was an interaction between pre-calving IMI and infusion of the teat sealant (P=0.06). This interaction was similar to that present in the final model for new infection with *Streptococcus uberis* i.e. that the use of a teat sealant decreased the risk of mastitis more in glands with an IMI pre-calving (RR=0.1, (95%CI 0.03-0.33), P<0.001) compared to glands with no IMI pre-calving (RR=0.33, (95%CI 0.21-0.51), P<0.001; Table 15). However the apparent difference in decreased risk is associated with the higher risk of clinical mastitis in glands with an IMI pre-calving (Figure 12).

The number of clinical mastitis cases from which no bacterial pathogen was isolated was higher in the glands treated with teat sealant than for the control glands (130/164 (79.3%) vs. 24/143 (16.8%), RR=4.7, (95%CI 3.3-6.9), P<0.001; Table 12).

Discussion

This study demonstrated that infusion of a teat sealant approximately 39 days pre-calving reduced the risk of new IMI, reduced post-calving prevalence of IMI and reduced the incidence of clinical mastitis. However, parenteral treatment with tylosin had no effect on cure of existing IMI, prevalence of IMI post-calving or on incidence of clinical mastitis.

Using a teat sealant is a preventive approach for heifer mastitis, and will reduce the number of animals requiring antibiotics for treatment of clinical mastitis post-calving. The hypotheses were that an antibiotic treatment would cure existing pre-calving IMI, infusion of an inert teat canal sealant would reduce new IMI and that their action would be additive in decreasing post-calving IMI and clinical mastitis. The infusion of

a teat sealant, approximately 39 days before calving reduced the risk of post-calving IMI by 65% and the incidence of clinical mastitis from which a bacterial pathogen was isolated by 74%. The likely mechanism of action of the teat sealant is that it is acting as a physical barrier within the teat canal, which reduces the risk of bacterial invasion, and hence reduces the risk of a new IMI (Meaney, 1977; Woolford et al., 1998). The effect of the teat sealant probably continues until the sealant is physically stripped (human) or sucked (calf) from the gland (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). This means the sealant is likely present in the canal during most of the high risk period, the few days before calving (Aarestrup and Jensen, 1997). Teat sealants have been used extensively at the end of lactation in cows, and the new dry period IMI rate was reduced 7 fold (Woolford et al., 1998), 3 fold (Berry and Hillerton, 2002) or by 80% (Godden et al., 2003).

In the current study, use of a teat sealant also decreased the risk of new IMI by any pathogen by 66% and by *Streptococcus uberis* by 73%. *Streptococcus uberis* is the most common pathogen isolated from clinical mastitis cases in all age groups of cows in New Zealand (Williamson et al., 1995; Pankey et al., 1996; McDougall, 1999), and is particularly a problem in heifers (Pankey et al., 1996; Compton et al, unpublished). The prevalence of *Streptococcus uberis* in the control group increased from 32/1036 (3.3%) approximately 39 days pre-calving to 98/1036 (9.5%) 0-5 days post-calving, illustrating the significant new IMI rate occurring with *Streptococcus uberis* over this time period. *Streptococcus uberis* was also the most commonly isolated pathogen from clinical mastitis cases in this study consistent with other New Zealand studies (Pankey et al., 1991; McDougall, 1999; McDougall, 2002; Compton et al., unpublished).

In the current study the use of a teat sealant decreased the risk of clinical mastitis from which a pathogen was isolated, but did not decease the risk of diagnosis of clinical mastitis. This is most likely due to the nearly 80% of glands infused with teat sealant, and subsequently diagnosed with clinical mastitis from which no pathogens were isolated. In contrast, only 17% of glands diagnosed with clinical mastitis that were not infused with teat sealant failed to isolate a pathogen. The higher occurrence of clinical mastitis cases from which no bacteria were isolated in the glands treated with the teat sealant is consistent with our previous work and is likely due to the presence of fragments of teat sealant in the treated glands post-calving (Parker et al, unpublished, chapter 4). The percentage of glands from which no bacteria were isolated in the control glands is consistent with previous studies (McDougall, 1999).

Previous studies have emphasised the need for aseptic technique in application of the teat sealant (Woolford et al., 1998; Huxley et al., 2002). In this study the technicians were fastidious about hygiene during this procedure and there were no adverse effects following the infusion of the teat sealant, such as clinical mastitis or sick heifers.

Infusion of intramammary antibiotics (both lactating and dry cow formulations) in heifers pre-calving (Trinidad et al., 1990b; Oliver et al., 1992; Oliver et al., 1997; Oliver et al., 2003), resulted in cure of existing IMIs and lead to a decrease in the prevalence of post-calving IMI, reduced clinical mastitis incidence and reduced somatic cell counts during the subsequent lactation. Anecdotal reports suggest that use of injectable antibiotics peripartum in heifers result in decreased post-calving IMI. Additionally injectable antibiotics have been shown to result in similar bacteriological cure rates as intramammary antibiotics for treating clinical mastitis in lactating cows

(McDougall, 1998; St Rose et al., 2003). Due to the seasonal calving pattern and large herd size in New Zealand, pre-calving treatment of heifers may need to be able to be applied to groups of >50 animals. Parenteral treatment may be more feasible than intramammary therapy under these management systems.

Tylosin treatment pre-calving did not result in cure of infected glands or reduce post-calving IMI. Possible reasons for this include the failure of the tylosin to reach concentrations above minimum inhibitory concentrations for sufficient duration, inherent or acquired resistance of the pathogens present to tylosin, or that cure did occur but that the gland became reinfected before post-calving sampling.

The dose and route of administration followed the New Zealand label directions for tylosin in lactating cows. Tylosin is a member of the macrolide antibiotic family and acts by inhibiting RNA-dependent protein synthesis by blocking translation at the ribosome. Macrolide and lincosamide compounds are generally highly effective against Gram-positive cocci (both *Staphylococcus* and *Streptococcus* spp.), moderately effective against *enterococcus* spp. and have a poor action against Gram-negative bacteria (Salmon et al., 1998). Tylosin is a weak base, and therefore reaches higher concentrations in milk than in serum due to the low pH of milk compared to serum (Ziv, 1980). In heifers pre-calving lactatogenesis is limited, hence it is likely that the pH of the secretion from the mammary gland is similar to serum. In this situation, the concentration of tylosin is unlikely to reach the same concentrations as in the lactating gland resulting in poorer curer rates.

The most commonly isolated species pre-calving was coagulase-negative staphylococcus, which has been reported to be resistant to some antibiotics (Hodges et al., 1984; Salmon et al., 1998). Salmon et al., (1998) reported that erythromycin (also a member of the macrolide antibiotic family) was effective against *Staphylococcus* and *Streptococcus* spp. isolated from the mammary glands of New Zealand and Danish dairy heifers. However, the range of efficacy was between MIC_{90}=0.13-64 ug/ml, suggesting that there was resistance within these bacterial species.

It has been hypothesised that the presence of an IMI pre-calving is an indicator that the teat canal is open, due to either the absence of a teat plug, or the poor quality of a teat plug (Compton et al, unpublished). If this is the case, then it is plausible that the tylosin may have in fact cured the infection that was present, but as the teat canal was open, reinfection occurred.

Use of antibiotics designed for lactating cows, in heifers pre-calving, does not meet the guidelines of the New Zealand regulatory authority (Agricultural Compounds in Veterinary Medicine). Antibiotic treatments would be expected to increase the risk of heifers producing milk with antibiotic residues in early lactation. Previous studies have found a range of concentrations of antibiotic present in milk post-calving, after pre-calving treatment with a selection of antibiotic therapies in heifers. Trinidad et al (1990b), found only 3% of quarters treated with a penicillin and dihydrostreptomycin dry cow therapy product 3 months before calving with residues within 3 days of calving. Oliver et al., (1992) found that 17% of composite quarter milk samples tested positive on the day of calving for antibiotic residues in heifers treated with lactating cow cloxacillin approximately 7 days pre-calving (0% following that), but that 85%, 28% and 0% were positive at 0, 3 and 10 days post-calving, respectively, following cephapirin treatment. One further study (Owens et al., 1994) reported no antibiotic

residues detected at 5 days post-calving following cephapirin dry cow therapy 10-14 weeks earlier. Antibiotic residues were not measured in this study, but the milk from all heifers was not sent for commercial supply until at least ten milkings after calving.

In the present study, pre-calving IMI was associated with an increased risk of post-calving IMI and clinical mastitis, in agreement with previous heifer study findings for *Streptococcus dysgalactiae* (Aarestrup and Jensen, 1997) and for all bacterial species (Parker et al, unpublished, chapter 4; Compton et al, unpublished). The presence of an interaction between pre-calving IMI and the efficacy of the teat sealant suggested that the teat sealant was more effective in glands with a pre-calving IMI. This may be explained using the hypothesis that the glands with an open teat canal and consequently having an existing pre-calving IMI, benefited more from the physical barrier of the teat sealant, because they were at increased risk of new infection with another pathogen compared to the non-infected glands. However, the increased risk of post-calving IMI and of clinical mastitis is more likely due to the effect of an IMI pre-calving. At univariate level the interaction was significant for all outcomes tested. Studies using teat sealant at drying off in lactating cows did not find an interaction between teat sealant and IMI at drying off in the risk of post-calving IMI and clinical mastitis (Huxley et al., 2002; Godden et al., 2003). However, Huxley et al, (2002) used teat sealant only in a subset of quarters (low SCC) and Godden et al, (2003) treated every quarter with teat sealant and antibiotics. Therefore the absence of an interaction between previous IMI and use of a teat sealant may have been affected by these factors.

Another possible explanation for the interaction between pre-calving IMI and teat sealant may be that the teat sealant is not inert but has antibiotic properties. Bismuth subnitrate has antibiotic action *in vivo,* but not *in vitro* against the human gastrointestinal bacteria *Helicobacter pylori* (Phillips et al., 2000). The mechanism of the antibiotic action of bismuth subnitrate is yet to be elucidated. However, in the current study the cure proportions were similar for each group, which suggests that teat sealant is not acting as an antimicrobial against these intramammary pathogens. The use of phenotypic bacteriological techniques as used in this study, may have underestimated the new infection and cure rates. This could occur if phenotypically similar, but genotypically dissimilar, isolates pre and post-calving were found, the gland would have been incorrectly defined as having a continuing rather than new infection or a cure over this time. Thus use of more accurate techniques reduces the tendency for misclassification to bias associations towards the null.

The effect of pre-calving IMI on new IMI in cows is uncertain. Some studies performed during lactation suggest that glands previously infected with minor pathogens, in particular coagulase-negative staphylococci, (Edwards and Jones, 1966; Rainard and Poutrel, 1988; Matthews et al., 1991) or *Corynebacterium bovis* were more resistant to new IMI than uninfected glands (Black et al., 1972; Rainard and Poutrel, 1988; Lam et al., 1997b). The relative protectiveness of pre-calving IMI with *Corynebacterium bovis* vs. coagulase-negative staphylococci is not well understood (Rainard and Poutrel, 1988; Lam et al., 1997a). Recent *in vitro* work has shown that *Staphylococcus chromogenes* taken from the teat apex of heifers consistently inhibited the growth of *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* (De Vliegher et al., 2004). Understanding the long-term effects of the presence of minor pathogens in the mammary gland requires further work, especially in primiparous cows. Based on the results from the current study, strategies intended to

reduce post-calving IMI and clinical mastitis in heifers should aim to reduce the prevalence of IMI pre-calving, as well as minimising new infections during the pre-calving period.

It has been shown that the risks of IMI for glands within a cow are not independent (Barkema et al., 1997), that is, clustering occurs. Statistical techniques to deal with non-independence of glands have been developed including the use of random effects terms in models (McDermott et al., 1994). In this study, the calculated ICC between glands was moderate. Mixed effects models, with random effect terms for herd and cow nested within herds, that accounted for correlation within cow and herd were used, with the correlation within cow (for post-calving IMI ICC=0.31) being far greater than correlation within herd (for post-calving IMI ICC=0.08). These ICCs are in similar proportion to each other as previously reported (Barkema et al., 1997). However, the degree of clustering within cow or within herds may be dependent on the bacterial pathogens present and the types of management systems operating. For example, it may be expected that contagious pathogens such as *Staphylococcus aureus* would have greater within cow clustering compared to environmental pathogens such as *Streptococcus uberis* (Barkema et al., 1997; Zadoks et al., 2001).

Conclusions

The findings of the current study show that teat sealant is an effective preventive against IMI and clinical mastitis in heifers, and presents a novel and practical option for farmers wishing to reduce mastitis in their heifers post-calving. The absence of interactions with the known covariates such as breed, herd, udder oedema, body condition score are important, as this suggests that the effect of teat sealant will be consistent for use in a wide range of herd and heifer types.

Table 10. Number and percentage (within treatment group) of bacterial species isolated from the mammary gland secretion collected on average 39 days before calving from 4164 glands from heifers subsequently treated with injectable tylosin (5g for 3 days at 24 hourly intervals), or infused with an intramammary teat sealant into all 4 glands (teat sealant), or treated with injectable tylosin (5g for 3 days at 24 hourly intervals) and infused with an intramammary teat sealant into all 4 glands (teat sealant and tylosin) or nothing (control).

Treatment groups	*Streptococcus uberis*	*Staphylococcus aureus*	CNS	Other Majors[2]	Other minors[3]	No growth[4]	Contaminants	No Sample	Total IMI	Total
Teat sealant (n)	27	4	108(19)[1]	7	5	851	27	18		1028
(%)	2.7	0.4	11.0	0.7	0.5	86.6	2.6	1.8	15.4	
Tylosin (n)	20	2	154(18)[1]	8	4	827	27	24		1048
(%)	2.0	0.2	15.4	0.8	0.4	82.9	2.6	2.3	18.9	
Teat sealant and Tylosin (n)	25	3	143(17)[1]	4(1)[1]	3(1)[1]	838	46	9		1052
(%)	2.5	0.3	14.3	0.4	0.3	84.1	4.4	0.9	17.9	
Control (n)	32	8	167(16)[1]	7	7(1)[1]	778	38	16		1036
(%)	3.3	0.8	17.0	0.7	0.7	79.2	3.7	1.5	22.5	
Total (n)	104	17	572(70)[1]	26(1)[1]	19(2)[1]	3294	138	67		4164
(%)	2.6	0.4	14.4	0.7	0.5	83.2	3.3	1.6	18.6	

[1]Brackets indicate the number of glands from which this species was isolated as the second isolate from that gland

[2] Other major pathogens = *Streptococcus dysgalactiae, Streptococcus agalactiae, Klebsiella spp., Pasturella spp., Enterococcus spp., Eschericia coli*

[3] Other minor pathogens = *Corynebacterium spp.*, yeast, gram-negative and gram-postive rods

[4] No growth=<3 colonies cultured, Bacillus, Fungi

Table 11. Number and percentages (within treatment group) of bacterial species isolates from the glands sampled by 0 and 5 days after calving (n=4164 glands) from heifers subsequently treated with injectable tylosin (5g for 3 days at 24 hourly intervals), or infused with an intramammary teat sealant into all 4 glands (teat sealant), or treated with injectable tylosin (5g for 3 days at 24 hourly intervals) and infused with an intramammary teat sealant into all 4 glands (teat sealant and tylosin) or nothing (control).

Treatment groups	*Streptococcus uberis*	*Staphylococcus aureus*	CNS	Other Majors[2]	Other Minors[3]	No growth[4]	Contaminants	Total IMI	Total
Teat sealant (n)	22	7	41(5)[1]	4(1)[1]	3	952	5		1028
(%)	2.2	0.7	4.0	0.4	0.3	93.1	0.5	7.5	
Tylosin (n)	112 (1)[1]	4	131(19)[1]	8(2)[1]	4(1)[1]	803	9		1048
(%)	10.8	0.4	12.6	0.8	0.4	77.3	0.9	24.9	
Teat sealant and Tylosin (n)	30	4	58(11)[1]	5(3)[1]	0	966	3		1052
(%)	2.9	0.4	5.5	0.5	0.0	92.1	0.3	9.2	
Control (n)	98	7	134(26)[1]	13(2)[1]	3(1)[1]	805	5		1036
(%)	9.5	0.7	13.0	1.3	0.3	78.1	0.5	24.7	
Total (n)	262	22	364(61)[1]	30(9)[1]	10(2)[1]	3526	22		4164
(%)	6.3	0.5	8.8	0.7	0.2	85.1	0.5	16.6	

[1]Brackets indicate the number of glands from which this species was isolated as the second isolate from that gland

[2] Other major pathogens = *Streptococcus dysgalactiae, Streptococcus agalactiae, Eschericia coli, Proteus*

[3] Other minor pathogens = *Corynebacterium spp.*, gram-negative and gram-postive rods

[4] No growth=<3 colonies cultured, Bacillus, Fungi

Table 12. Number and percentage of bacterial species isolated from glands diagnosed with clinical mastitis 14 days post-calving within treatment group from heifers subsequently treated with injectable tylosin (5g for 3 days at 24 hourly intervals), or infused with an intramammary teat sealant into all 4 glands (teat sealant), or treated with injectable tylosin (5g for 3 days at 24 hourly intervals) and infused with an intramammary teat sealant into all 4 glands (teat sealant and tylosin) or nothing (control).

Treatment groups	*Streptococcus uberis*	*Staphylococcus aureus*	CNS	Other Majors[2]	No growths[3]	Contaminants	Total
Teat sealant (n)	9	3	4(1)[1]	0	45	0	60
(%)[4]	15.0	5.0	6.7	0.0	75.0	0.0	
Tylosin (n)	51	1	10(1)[1]	3	10	0	74
(%)[4]	68.9	1.4	13.5	4.1	13.5	0.0	
Teat sealant and Tylosin (n)	8	2	7(2)[1]	1	85	3	104
(%)[4]	7.9	2.0	6.9	1.0	84.2	2.9	
Control (n)	39	2	10(2)[1]	7(1)[1]	14	0	69
(%)[4]	56.5	2.9	14.5	10.1	20.3	0.0	
Total (n)	107	8	31(6)[1]	11(1)[1]	154	3	307
(%)[4]	35.2	2.6	10.2	3.6	50.7	1.0	

[1] Brackets indicate the number of glands from which this species was isolated as the second isolate from that gland

[2] Other major pathogens = *Streptococcus dysgalactiae, Eschericia coli*

[3] No growths=<3 colonies cultured, Bacillus, Fungi

[4] % of clinical mastitis isolates within the treatment group

Table 13. Relative risk (RR) and 95% confidence intervals (CI) and probability values (P) of post-calving IMI for heifers treated with injectable tylosin (5g for 3 days at 24 hourly intervals) or infusion of an intramammary teat sealant into all 4 glands, approximately 39 days pre-calving.

						95% CI		
Effect	Category	Estimate	Standard Error	t value	RR	Lower	Upper	P
Intercept		0.44	0.74	0.6				0.55
Teatseal		-1.05	0.11	-9.13	0.35	0.28	0.44	<0.001
Tylosin		0.02	0.11	0.2	1.02	0.82	1.27	0.84
Pre-calving IMI		1.18	0.07	16.4	3.25	2.82	3.74	<0.001
Breed	Friesian	0.28	0.16	1.74	1.33	0.97	1.82	0.08
	Jersey	-0.41	0.16	-2.49	0.67	0.48	0.92	0.01
	Crossbred[1]							
Body condition score	<4.5	-0.52	0.28	-1.87	0.60	0.35	1.03	0.06
	4.5	-0.68	0.23	-2.88	0.51	0.32	0.81	0.04
	5	-0.41	0.20	-2.08	0.66	0.45	0.98	0.04
	5.5	-0.24	0.20	-1.17	0.79	0.53	1.17	0.24
	>5.5[1]							
Calving to sampling (days)[2]		-0.27	0.05	-5.79	0.76	0.70	0.84	<0.001
Min teat height (cm) [3]		-0.04	0.01	-2.55	0.96	0.94	0.99	0.01
Herd[4]		0.08						
Cow nested within herd[4]		1.6						

[1] Reference category

[2] Interval between calving and post-calving sampling (0-5 days)

[3] Minimum teat end height from the ground (cm)

[4] Random effect terms in model

Table 14. Relative Risk (RR) and 95% confidence intervals (CI) for new intramammary infection (IMI) with any pathogen between approximately 39 days pre-calving and 0-5 days post-calving in heifers treated with injectable tylosin (5g for 3 days at 24 hourly intervals) or infusion of an intramammary teat sealant into all 4 glands, approximately 35 days pre-calving.

					95% CI		
Effect	Estimate	Standard Error	t value	RR	Lower	Upper	P value
Intercept	-0.55	0.74	-0.74				0.47
Teatseal	-1.09	0.13	-8.54	0.34	0.26	0.43	<0.001
Tylosin	0.07	0.12	0.6	1.08	0.84	1.38	0.55
Calving to sampling (days)[1]	-0.26	0.05	-4.95	0.77	0.70	0.86	<0.001
Min teat height (cm)[2]	-0.03	0.01	-1.98	0.97	0.95	1.00	0.05
Herd[2]		0.16					
Cow nested within herd[2]		1.90					

[1] Interval between calving and post-calving sampling (0-5 days)

[2] Minimum teat end height from the ground (cm)

[3] Random effect terms in model

Table 15. Relative risk (RR) and 95% confidence interval (CI) of glands being diagnosed with clinical mastitis up to 14 days post-calving from which bacterial pathogens were isolated in heifers treated with injectable tylosin (5g for 3 days at 24 hourly intervals) or infusion of an intramammary teat sealant into all 4 glands, approximately 39 days pre-calving.

					95% CI		
Effect	Estimate	Standard Error	t value	RR	Lower	Upper	P value
Intercept	-4.75	0.18	-26.06	0.01			<0.001
Teatseal	-1.20	0.23	-5.28	0.30	0.19	0.47	<0.001
Tylosin	0.01	0.21	0.03	1.01	0.66	1.52	0.98
Pre-calving IMI	0.84	0.09	9.07	2.31	1.92	2.76	<0.001
Teatseal*Pre-calving IMI	-0.38	0.21	-1.86	0.68	0.46	1.02	0.06
Herd[1]		0.13					
Cow nested within herd[1]		4.2					

[2] Random effect terms in model

Figure 9. Prevalence of glands with intramammary infection (IMI) post-calving (%) by day at sampling.

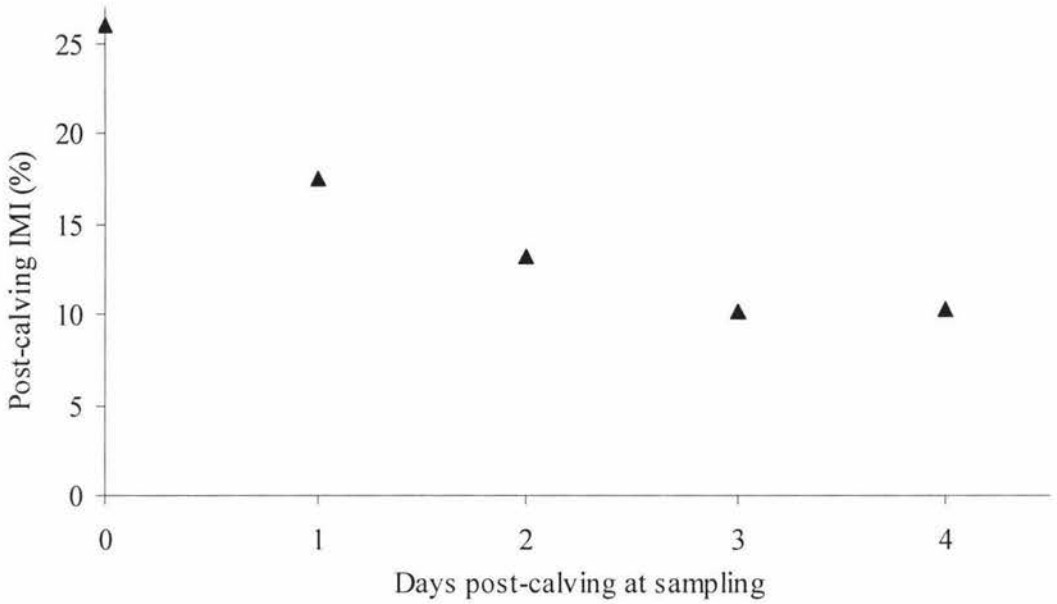


Figure 10. Raw gland level prevalence of post-calving intramammary infection (IMI; %) (dotted) and relative risks (RR) of post-calving IMI calculated from the final model (solid line), by body condition score day 0-5 post-calving.

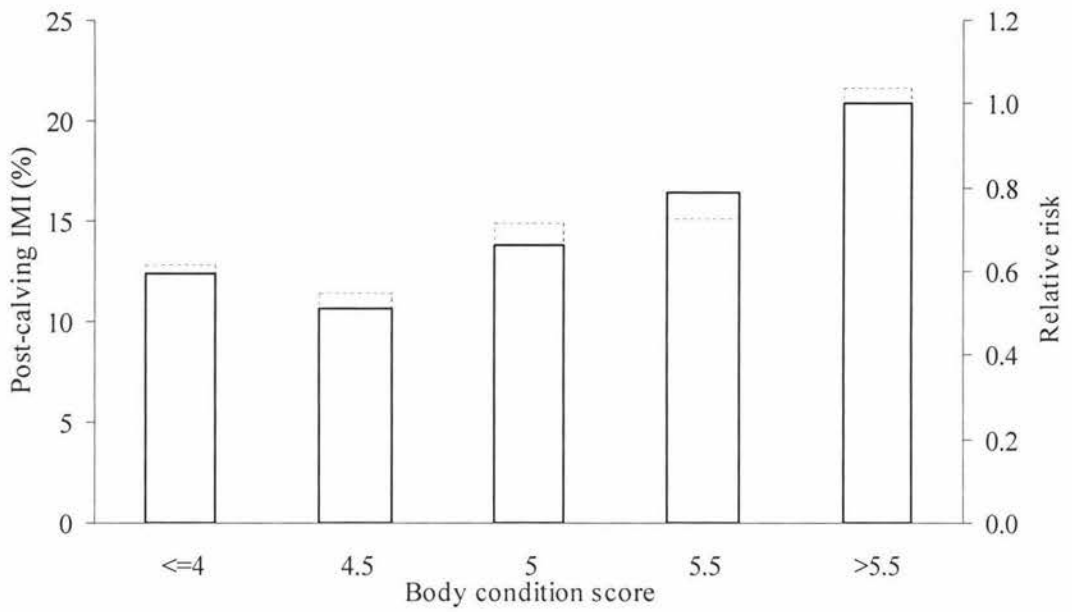


Figure 11. Incidence of new intramammary infection (IMI) (%) of any pathogen by minimum teat height for all glands, with a linear trend line fitted through the raw data.

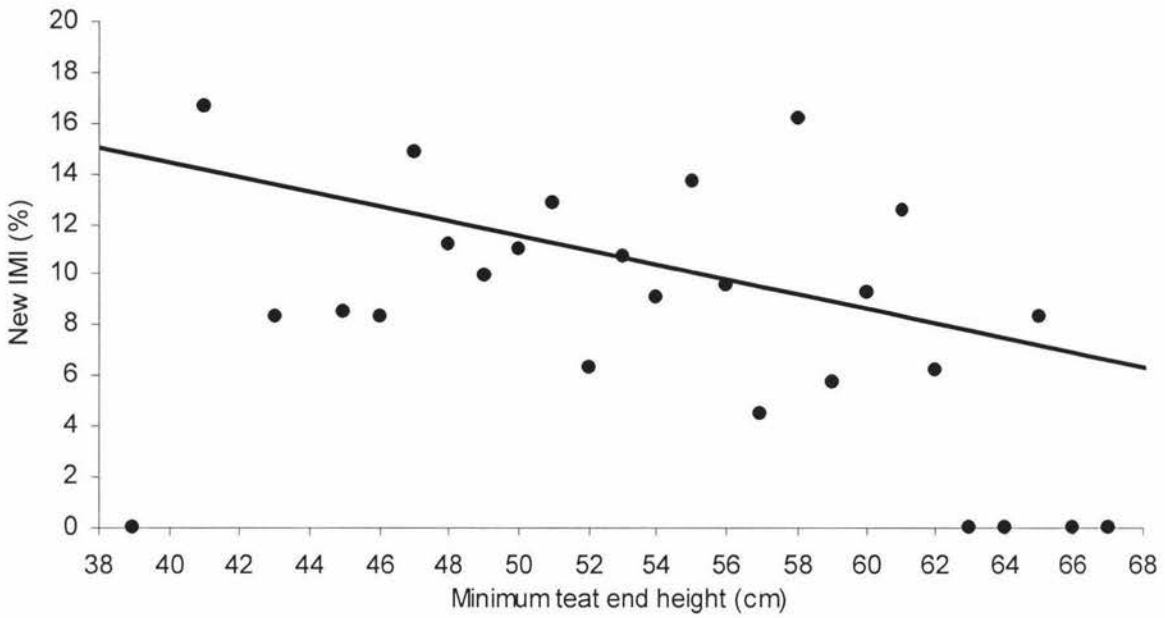
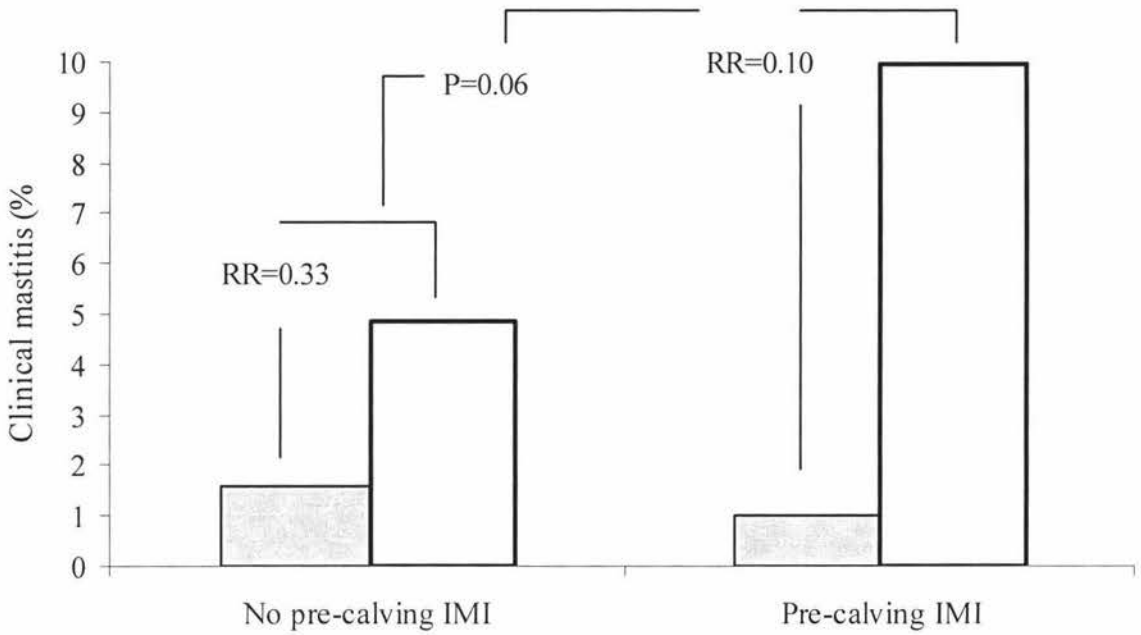


Figure 12. Raw gland level incidence of clinical mastitis (%) occurring within the first 14 days after calving that cultured any pathogen by treatment group (infusion of all glands with teat sealant (grey) or untreated glands (white)), blocked by the pre-calving IMI status for each gland.



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Chapter 6 The effects of a teat sealant and/or and injectable antibiotic pre-calving in primiparous heifers on subclinical and clinical mastitis and somatic cell counts

Abstract

AIMS:

The aims of this study were to investigate the heifer-level effects of infusion of a bismuth subnitrate teat-canal sealant and/or and injectable antibiotic on the prevalence of post-calving IMI, incidence of clinical mastitis in the first two weeks post-calving, individual cow somatic cell counts (SCC) and risk of premature removal from the herd.

MATERIALS AND METHODS:

Heifers (n=1067) in 30 seasonally calving, pasture-fed dairy herds were randomly assigned to one of four treatment groups (no treatment; three injections of 5g of tylosin antibiotic; infusion of a teat sealant into all four quarters; three injections of 5g of tylosin antibiotic and infusion of teat sealant into all four quarters). Mammary gland secretion samples were collected from each quarter of every heifer before treatment. Heifers within a herd were enrolled on one calendar day, on average 27 days before the planned start of the seasonal calving period. Duplicate milk samples were collected from each gland within five days after calving for bacterial culture, and from glands the herd-owners diagnosed as having clinical mastitis, before treatment. Milk yield and somatic cell count (SCC) data was collected on four occasions across the subsequent lactation. The relative risk of the post-calving infection, clinical mastitis and culling were calculated using Mantel Haenszel and logistic regression analyses, and the effect of treatment on SCC was measured using a generalised linear model.

RESULTS:

Infusion of the teat sealant reduced the risk of post-calving IMI due to any pathogen by 67%, of clinical mastitis by 25% and decreased SCC by approximately 1000 cells/ml. Teat sealant had no effect on risk of culling. Tylosin had no effect on prevalence of IMI, incidence of clinical mastitis, SCC or risk of culling.

CONCLUSIONS:

Infusion of a teat sealant pre-calving provides a useful tool for reducing the risk of subclinical and clinical mastitis in heifers.

CLINICAL RELEVANCE

Currently there are few management strategies that can be recommended to farmers where there is a high incidence of clinical mastitis post-calving in heifers. Teat sealant provides a realistic option to decrease clinical mastitis in pasture-fed dairy heifers.

KEYWORDS: mastitis, Streptococcus uberis, heifer, teat sealant, antibiotics

ABBREVIATIONS:

IMI Intramammary infection
ICC Intraclass correlation
RR Relative risk
OR Odds ratio
SCC Somatic cell count

Introduction

International and local research has shown that high individual somatic cell counts (ICSCCs) and/or clinical mastitis, occurring early in the first lactation results in lower production during the first lactation (De Vliegher et al 2005), long term production losses (Woolford et al 1983; 1984), increased incidence of clinical mastitis in the following lactation and increased risk of premature removal from the herd (Myllys and Rautala 1995; Rupp et al 2000; Rupp and Boichard 2000). The incidence of clinical mastitis, especially around the time of calving, has been shown to be higher in heifers compared to cows in a number of studies (Hogan et al 1989; Barkema et al 1998; McDougall 1999). However, the reasons for this are not clear.

Preventive measures for mastitis in dairy heifers are not well described. Existing mastitis management programmes such as the SAMM plan and the 'Five-Point Plan' focus primarily on multiparous animals, and on reducing risk of transmission and shortening the duration of infection of the 'contagious' pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Smith et al 1967; Woolford et al 1995). The efficacy of such programmes for reducing risk of IMI before calving in heifers and in reducing the risk of *Streptococcus uberis* mastitis, the most common isolate under New Zealand pasture-based production systems (Pankey et al 1996; McDougall 1999), remains largely untested.

Bacteria have been isolated from the mammary gland of heifers as early as 9 months pre-calving (Trinidad et al 1990a) and the prevalence of infection increases as calving approaches (Oliver and Mitchell 1983; Aarestrup and Jensen 1997). European and American pre-calving prevalence's of IMI range from 20% to 97% (Meaney 1981; Trinidad et al 1990a; Myllys 1995). Under New Zealand systems, the gland level prevalence of pre-calving intramammary infection (IMI) is 16.5% (Parker et al unpublished, chapter 4).

The effect of pre-calving IMI on the risk of a new IMI around calving is unclear. Some studies in cows have shown that IMI with a minor pathogen may reduce the prevalence of IMI with major pathogens at calving and in early lactation (Rainard and Poutrel 1988; Lam et al 1997; Green et al 2002). However, other studies have found that existing IMI with a minor pathogen increases the risk of IMI with another pathogen (Pankey et al 1985; Hogan et al 1988). If pre-calving IMI is a risk factor for post-calving IMI and clinical mastitis, then reduction in the prevalence of IMI in the last trimester may lead to a reduction in post-calving IMI and clinical mastitis.

Intramammary infusion of antibiotics pre-calving has been shown to reduce the prevalence of IMI post-calving, reduce the incidence of clinical mastitis and decrease somatic cell counts (SCC) during the subsequent lactation (Oliver et al 1992; Oliver et al 1997; Oliver et al 2003). However, under seasonal calving systems, where individual calving dates of heifers are not known and in larger herds, repeated infusion of all glands of all heifers may present significant logistical problems and present increased risk of trauma to staff and heifers. Intramuscular administration of antibiotic therapy may be more practical. Parenteral antibiotic therapy of clinical mastitis has been successfully used in lactating cows (Ziv and Storper 1985; McDougall 1998; St Rose et al 2003). In a small study Williamson (2002) demonstrated that injection of penicillin once or twice within 7 days before

anticipated calving significantly reduced the percentage of infected quarters at the first milking, but that there was no significant difference in the incidence of clinical mastitis. Bryan and Friton (2004) reported that treatment with intramuscular penethamate in heifers pre-calving decreased the number of heifers with clinical mastitis at calving. The macrolide antibiotic tylosin, has been demonstrated *in vitro* to have efficacy the common Gram-positive mastitis pathogens (Salmon et al 1998) and has been used effectively to treat clinical mastitis in dairy cows (McDougall et al, unpublished).

Infusion of an inert 'teat sealant' into the teat canal has been shown to reduce the risk of new IMI during the dry period of lactating cows with resultant reduced prevalence of IMI after calving and reduced incidence of clinical mastitis (Meaney 1977; Woolford et al 1998; Berry and Hillerton 2002; Huxley et al 2002). In a pilot study, infusion of a teat sealant approximately 4-6 weeks before calving decreased the prevalence of IMI of *Streptococcus uberis* in heifer's quarters 0 to 5 days postpartum by 84% (Parker et al., unpublished, chapter 4). However, that study had limited statistical power and used a within animal treatment model so that heifer level estimates of treatment effects on post-calving IMI, incidence of clinical mastitis, milk production, somatic cell count (SCC) or premature removal from the herd could not be estimated.

Therefore the hypotheses of this study were that the infusion of a teat sealant and/or parenteral treatment with tylosin approximately 30 days before the planned start of calving in seasonal dairy herds in heifers would result in:

1. A reduction in the prevalence of heifer level IMI between day 0 and 5 post-calving,
2. A reduction in the proportion of heifers with clinical mastitis in one or more quarter during the 14 days post-calving,
3. A reduction in the individual animal SCC across lactation,
4. A reduction in the number of heifers culled during or at the end of lactation, and
5. An increase in the milk yield across lactation.

Material and Methods

Heifers (n=1067) from 30 spring calving, pasture-fed dairy herds were enrolled. The herds were located in the Waikato region of the north island of New Zealand. Between 18 and 48 heifers were enrolled from each herd. The herds ranged in size from 214 to 810 cows (median = 310). Herds were selected on the basis that herd-owners would undertake 4 milk production recordings ("herd tests") in the season of the study, would provide animal level data to an electronic database (Livestock Improvement Corporation, Hamilton), farmed within a radius of 30 km of Morrinsville Animal Health Centre, maintained high quality records and agreed to comply with the study protocol.

A priori a sample size of 1000 heifers was calculated based on the assumption that the gland level prevalence of IMI post-calving would be decreased by the teat sealant and antibiotic treatment using an absolute effect size of 0.05 (W), which is a measure of the magnitude of the Chi-square of the difference, (Hintze, 2001, NCSS and PASS). A preliminary study estimated gland level prevalence of IMI to be ~ 16% pre-calving and 12% post-calving. Teat sealant reduced the prevalence of post-calving IMI from

~ 12.1% to 9.7% and cumulative incidence of clinical mastitis from ~ 6.9% to 4.5% (Parker et al unpublished, chapter 4). Hence, to demonstrate a 5% difference (with a 95% confidence limit (α=0.05) and 80% power (β=0.2)) in clinical mastitis incidence, with the control group having an incidence of 10%, then 474 glands (i.e. ~ 120 cows/treatment group) were required.

The design is a 2 by 2 factorial design and gland is nested within cow resulting in some correlation within heifer (i.e. glands are not entirely independent so the variance estimate needs to be adjusted for the intraclass correlation). Thus, the number of heifers enrolled was doubled to account for the non-independence of glands and the loss to follow up of some animals.

Heifers in each herd were enrolled on one calendar day, at a median of 27 days (SD=4) before the planned start of the seasonal calving period. The heifers calved between 5 and 127 days after enrolment (median=39, SD=16.6). Treatment was assigned within herd in a 2 by 2 factorial arrangement at heifer level. Each heifer was randomly assigned to one of five treatment groups using a random number generator function in "R: A language and environment for statistical computing" (R Development Core Team 2005) version 2.2.0. The number of heifers available in each herd and the number required for this study determined the sample size from each herd. Heifers were assigned to two control groups, however one of the control groups was used only in an observational study to be reported elsewhere (Compton et al., unpublished).

Before treatment, each teat end was scrubbed with a cotton wool pledget moistened in 70% methanol and a gland secretion sample collected aseptically. No secretion was discarded before collection due to the small volume of secretion present in most glands. Where no secretion could be collected from a gland it was recorded as a missing sample (n=99 glands). Following sampling, all four glands within a heifer were either infused with 2.6g of bismuth subnitrate following teat end scrubbing (n=268; Teat Seal, Pfizer Animal Health NZ Ltd, Auckland, New Zealand), or a heifer was injected intramuscularly with 5 g of tylosin for 3 days at approximately 24 hour intervals (n=268; Tylan 200, Elanco Animal Health, Manukau City, New Zealand), or all four glands were infused with the teat sealant and the heifer was treated with 5 g of tylosin i.m. daily for 3 days (n=266), or they were left as untreated control (n=265). The tip of the teat sealant cannula was inserted approximately 3 mm into the teat canal for infusion. Following sampling or infusion, the teats ends were spayed with an antiseptic solution containing 0.5% effective iodine. Technicians infused the teat sealant and administered the first treatment of tylosin. They left the remaining two treatments in labelled 25 ml syringes, for farm staff to administer.

The body condition score on a 1-10 scale in half score increments (Roche et al 2004), the udder and escutcheon hygiene score from 0-3 (Schreiner and Ruegg 2003), the length of the tail of each heifer, (ordinal scale: 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); and the presence of udder oedema (as determined by digital pressure in the caudal aspect of the udder resulting in the presence of a depression for > 30 secs) were recorded for each animal at enrolment and again at the post-calving sampling. The distance from the ground to the lowest teat end (in cm) was recorded at the post-calving sampling.

Within 5 days of calving (range 0 to 5 days after calving; median=2 days), technicians collected duplicate milk samples (~ 20 ml each) from each gland, following aseptic preparation of the teat end and discard of the first 3 strippings. Herd-owners were asked to diagnose clinical mastitis on the basis of presence of visible changes (e.g. clots or blood) in the milk composition or swelling and/or pain in the gland. Technicians collected duplicate milk samples (~20 ml each) from each gland diagnosed with clinical mastitis where it occurred within 14 days of calving, following aseptic preparation of the teat end and discard of the first 3 strippings. Where clinical mastitis was diagnosed on, or before, the scheduled day 0-5 post-calving visit, all glands including the gland with clinical mastitis were sampled and the bacteriology results were included in both the post-calving day 0-5 analysis (post-calving IMI) and the clinical mastitis analysis.

Bacteriology

Pre-calving secretion samples were processed for bacteriology within 24 h of collection following storage at 4°C. Post-calving and clinical mastitis milk samples were stored at 4°C before processing, which occurred within 24 h of collection.

Microbiological procedures, diagnosis of IMI, and categorisation of results were undertaken using standard methodology with minor modifications (National Mastitis Council 1999, USA). Ten µl of milk was streaked onto a quadrant of a 5% sheep blood agar plate containing 0.1% esculin (Fort Richard, Auckland, New Zealand) following gentle inversion at room temperature, and incubated at 37°C for 48 h. For post-calving, which were collected in duplicate, both samples were plated onto a quadrant of the same plate for easy comparison of results. The genus of bacteria was provisionally determined on the basis of colony morphology, Gram stain, haemolysis pattern, catalase test and esculin reaction. Gram-positive, catalase-positive isolates were further tested using a tube-coagulase test, and coagulase-positive isolates were defined as *Staphylococcus aureus* and coagulase-negative isolates as coagulase-negative staphylococci. Gram-positive, catalase-negative isolates were CAMP tested and esculin-positive and CAMP-positive or negative isolates were defined as *Streptococcus uberis*, esculin-negative and CAMP-negative isolates as *Streptococcus dysgalactiae* and esculin-negative and CAMP-positive isolates as *Streptococcus agalactiae*. Gram-negative rods were sub-cultured on MacConkeys agar, had an oxidase test performed and were cultured in triple sugar iron agar and simmons citrate agar. Gram-negative rods that could be identified were recorded as genus level, while those not identified were recorded as gram-negative rods. Gram-positive rods that could be identified with simple procedures were identified and recorded e.g. *Corynebacterium* spp.

A gland was defined as infected where 3 or more of each of 1 to 2 colony morphology types was present. *Bacillus spp.*, Fungi or <3 colonies present were defined as "no growths". For the duplicate, post calving and clinical mastitis samples, both of the duplicate samples were required to isolate the same pathogen, unless one sample was contaminated in which case the other sample result was reported. A milk sample was defined as contaminated if >2 distinct colony types were present on culture, and these results were reported but not included in further analyses.

The SCCs were determined using flouro-optic techniques (Foss 5000, LIC Riverlea, Hamilton, NZ) following preservation of the sample with bronopol.

Data Handling
Heifer level data including calving date, breed, SCC and milk yield at herd test were retrieved from the national database (Livestock Improvement Corporation, Hamilton, New Zealand). Cow removal date and reason ("culling") and disease records were collected from the herd-owners during, and at the end of, lactation.

Results from samples and measurements that were collected >5 days post-calving that were intended for use as routine samples (n=13 heifers) or for the data from clinical mastitis cases that occurred >14 days after calving were discarded from analyses (n= 8). Twelve heifers failed to calve and one heifer was not sampled post-calving. Thirty-four heifers were lost to follow up (Table 16). The data analysis was conducted on 980 heifers.

Data Analysis
Descriptive statistics at heifer level were performed.

Dependent variables included the presence of IMI in at least one gland when sampled 0 to 5 days postpartum (i.e. "post-calving IMI"; dichotomous), diagnosis of clinical mastitis in one or more glands within 14 days of calving ("clinical mastitis"; dichotomous), failure to isolate a bacteria from the clinical mastitis sample ("no growth"; dichotomous), removal from the herd on or before the end of lactation ("culling"; dichotomous), and the log 10 SCC (continuous) and estimated total milk solids production (i.e. milk fat and milk protein; Kg/heifer/day; continuous) at each herd test in the lactation.

The main effects of infusion of the teat sealant (yes/no; binomial) and treatment with tylosin (yes/no; binomial) were included in all models. Other independent variables examined during the model building process included breed (defined as the predominant parentage breed if greater than $11/16^{th}$ (Friesian or Jersey), and all other breeds were classified as crossbreds; categorical), pre-calving IMI at heifer level (present if $\geq$ 1 gland with an IMI/absent; binomial), interval between treatment and calving (continuous variable, days), interval between calving and post-calving sampling (continuous variable, 0-5 days), interval between calving and each herd test (continuous variable, days), the minimum height (in cm) of the teat end from the ground (continuous variable), presence of udder oedema pre or post-calving (binomial; present or absent), body condition score pre-calving and at 0-5 days post-calving (categorised into 5 groups <4.5, 4.5, 5, 5.5, >5.5), and udder hygiene score post partum (ordinal, 0 to 3, scale).

For the categorical dependant variables, bivariate associations between independent and dependent variables were examined using chi-square analysis for the categorical variables, and crude relative risks (RR) were calculated. The Mantel-Haenszel procedure was used to stratify the data by covariates such as pre-calving IMI and adjusted RRs were calculated. If the main effects were not significant in the bivariate analysis no further modelling was undertaken and the crude RRs were reported. Associations between continuous independent variables and prevalence of IMI and clinical mastitis were examined graphically and using Pearson's correlation. Predictor

variables from the bivariate and stratified analyses associated with the binomial outcome variables (i.e. P<0.2) were manually added in a step-wise manner to generalised mixed log binomial models (GLIMMIX, SAS v 9.1, 2004). Independent variables were offered to the model in descending order of significance at the bivariate level. The log link was used as exponentiation of the coefficients from these models provides the RR. This avoids the overestimation of effects, which occurs where odds ratios (OR) are calculated by exponentiation of the coefficients from models where the commonly used logit link function is used and where the prevalence of disease is >10% (Dohoo et al 2003; McNutt et al 2003).

As cows within herds may not be biologically independent, the degree of correlation of results between heifers within herds needs to be accounted for in modelling (McDermott et al 1994; Barkema et al 1997). Including a random effect term for herd in the final models accounted for this.

Milk yield and log 10 SCC were measured 1 to 4 times for each heifer during the study. If a heifer had missing herd test data the herd test information for the other three tests was still included in the analysis. These measurements within a heifer are not independent and it is likely that data from temporally adjacent herd tests are more closely related than measurements separated by a greater time interval. To account for this correlation, mixed repeated measures models with first order autoregressive correlations were developed. Autoregressive correlation was used as it provided the best model fit (smallest model deviance) and was the most biologically plausible adjustment matrix. The interval from calving to herd test and this interval squared were included as covariates as they significantly improved the model fit.

The smallest –2log likelihood (model deviance), the type 3 sums of squares for each variable, and the coefficients and error terms of the main effects were used to determine which variables remained in the final models. Variables were included into the models if they reached significance (P<0.05). Biologically meaningful interactions were tested including teat sealant by tylosin, teat sealant by pre-calving IMI status, teat sealant by calving to sampling time and teat sealant by udder oedema post-calving. If the interaction was not significant and it did not alter the coefficient of the main effects by >10% then the interaction was not included in the model.

A number of covariates remained in the model, despite them not being confounders (i.e. they did not alter the main effect coefficients by >10%), because they explained a large amount of variation in the model, improved the model fit and it made biological sense to include them. Model diagnostics were performed, including plotting residuals against the predicted values and examining outliers (i.e. those values >2 SD from the expected values).

Statistical significance was defined as P≤0.05, and confidence intervals (CI) reported are for a 95% range of values. Data was recorded in a Microsoft Access database, and statistical analyses were carried out using SAS version 9.1 (www.sas.com).

Results

There were no differences between treatment groups in terms of calving date, breed, condition score, post-calving oedema, teat height, herd test to calving interval or treatment to calving interval (Table 16).

The pre-calving heifer level prevalence of IMI was 36.4% and did not differ between treatment groups (Table 16). Pre-calving IMI was present in 1, 2, 3 and 4 glands of 18.1%, 10.5%, 4.9% and 2.9% of heifers, respectively. Coagulase-negative staphylococcus was isolated from one or more quarters in 32.4% of heifers pre-calving. *Streptococcus uberis* was isolated from one or more quarters in 8.0% of heifers and *Staphylococcus aureus* from one or more quarters in 1.4% of heifers.

The post-calving prevalence of IMI in the control heifers was 48.6%. Post-calving IMI was present in 1, 2, 3 and 4 glands of 19.0%, 7.8%, 4.7% and 2.1% of heifers, respectively. Coagulase-negative staphylococcus was isolated from one or more quarters in 22.0% of heifers post-calving. *Streptococcus uberis* was isolated from one or more quarters in 17.1% of heifers and *Staphylococcus aureus* from one or more quarters in 1.8% of heifers.

The prevalence of clinical mastitis was 21.4% in the control heifers in the period up to 2 weeks post-calving with *Streptococcus uberis* the most common pathogen isolated from these cases (49.1% of all clinical mastitis cases). Across all treatment groups 13.6% of heifers were recorded as removed (culled or died) either during or at the end of the lactation (Table 16).

Teat sealant decreased the risk of post-calving IMI by 57% (230/486 (47.3%) vs. 101/494 (20.4%), RR = 0.43 (95% CI 0.35-0.52), P<0.001; Table 18). Post-calving IMI decreased with increasing interval between calving and sampling (P<0.001) and increased with presence of IMI pre-calving (P<0.001; Table 18).

Teat sealant decreased the risk of clinical mastitis (99/486 (20.4%) vs. 76/494 (15.4%) for control and teat sealant group respectively, RR=0.75 (95%CI 0.58-0.96), P=0.02; Table 19). Clinical mastitis prevalence decreased with increasing minimum teat height (P=0.002) and increased with increasing treatment to calving interval (P=0.002; Table 19).

Using bivariate analysis the number of clinical mastitis cases from which no pathogen was isolated, was higher in the teat sealant treated heifers compared to the non-teat sealant treated heifers (53/494 (10.7%) vs. 22/486 (4.5%), RR= 2.4 (95%CI 1.5-3.8), p<0.001; Table 16). The number of multiple quarters within a heifer that were diagnosed with clinical mastitis but that isolated no bacteria was higher in the heifers treated with teat sealant compared to those not treated with teat sealant (Table 17).

Heifers that were infused with a teat sealant had a lower log10 SCC (P=0.08, Table 20) than untreated heifers and this was consistent across lactation, as there was no time by treatment interaction (p=0.85; Figure 13). Friesians had a higher SCC than crossbreds (P<0.001) and increasing the herd test to calving interval was associated with changing SCC (P<0.001).

Tylosin had no effect on the prevalence of post-calving IMI (RR=1.02, (95%CI 0.90-1.15), p=0.78; Table 18), on clinical mastitis incidence (RR=0.94 (95%CI 0.73-1.2), p=0.61; Table 19), or on SCC during lactation (P=0.32; Table 20).

There was no effect of pre-calving treatment with teat sealant or tylosin on milk production throughout lactation (p=0.82; Table 16). There was no effect of either teat sealant on the number of heifers prematurely removed (culled) from the herd (68/486 (14%) vs. 66/494 (13.4%), p=0.66; Table 16), or of tylosin on the number of heifers culled.

Discussion

This study has demonstrated that infusion of a teat sealant approximately 39 days pre-calving reduces the prevalence of post-calving IMI by 57%, reduces the incidence of clinical mastitis by 25% and leads to a decrease in SCC by ~ 1000 cells/ml across lactation. However, parenteral treatment with tylosin had no effect on prevalence of IMI post-calving, incidence of clinical mastitis or on SCC.

The teat sealant is likely to be acting as a physical barrier within the teat canal reducing the probability of bacterial invasion, hence reducing the risk of a new IMI and the prevalence of IMI around calving. In multiparous cows, infusion of a teat sealant at the end of lactation results in a 7 fold (Woolford et al 1998), 3 fold (Berry and Hillerton 2002) or 80% (Godden et al 2003) reduction in new IMI across the non-lactating period. The protective effect of the teat sealant is likely to continue until the sealant is physically stripped (human) or sucked (calf) from the gland (Woolford et al 1998; Berry and Hillerton 2002; Huxley et al 2002). Hence, the sealant may still be present in the teat canal during though the final few days of gestation, potentially a period of high risk of new IMI (Aarestrup and Jensen 1997).

In a pilot study (Parker et al, unpublished, chapter 4), and the gland level analysis of this data (Parker et al, unpublished, chapter 4) the use of teat sealant did not decrease the overall risk of clinical mastitis, but it did reduce the risk of clinical mastitis from which any pathogen was isolated by 69% in the pilot study and by 74% in the gland level analysis. The current study was consistent with the pilot study in that, a higher proportion of clinical mastitis milk samples from glands previously treated with the teat sealant than those not treated with teat sealant had no bacteria isolated. It is likely that the presence of 'flecks' of the teat sealant were interpreted as clinical mastitis by herd-owners. These 'flecks' of teat sealant have been reported to be present in the milk for up to three weeks in other studies (Huxley et al 2002). However, this does not explain why the overall effect of teat sealant on clinical mastitis incidence was not significant in the gland level analysis of this data set. The number of glands with clinical mastitis that isolated no bacteria could explain this apparent contradiction. At the gland level the number of "no growths" was more than five times higher in the teat sealant treated glands compared to the control glands (Parker et al unpublished, chapter 4). However, at heifer level the number of "no growths" in the heifers treated infused with teat sealant was only double that of the heifers that did not get infused with teat sealant. The number of heifers with clinical mastitis in multiple quarters with no bacteria isolated was 24 in the teat sealant treated groups compared to 2 heifers in the non-teat sealant treated groups. This explains the discrepancy between the numbers of "no growths" in the data at quarter vs. heifer level. The increase in

multiple quarters with clinical mastitis that isolated no bacteria may be associated with farmer diagnostic practices i.e. once a heifer is diagnosed with clinical mastitis in one gland farmers may be more likely to identify the other glands as clinical mastitis also (sensitivity increases).

Infusion of intramammary antibiotics (both lactating and dry cow formulations) in heifers pre-calving reduces the prevalence of post-calving IMI, reduces clinical mastitis incidence and reduces SCC during lactation (Trinidad et al 1990b; Oliver et al 1992; Oliver et al 1997; Oliver et al 2003). Anecdotal reports suggest that use of injectable antibiotics peripartum in heifers result in decreased post-calving IMI (Williamson 2002). Additionally, injectable antibiotics have been shown to result in similar bacteriological cure rates as intramammary antibiotics for treating clinical mastitis in lactating cows (McDougall 1998; St Rose et al 2003). Parenteral antibiotic treatment was evaluated in the current study, as it was perceived were the approach to work, that injections would be easier for herd-owners to undertake than intramammary infusion. However, tylosin did not decrease the risk of post-calving IMI, incidence of clinical mastitis or decrease SCC. Possible reasons for this include the failure of the tylosin to reach concentrations above minimum inhibitory concentrations (MIC) for sufficient duration, inherent or acquired resistance of the pathogens to tylosin, or that cure did occur but that the gland was reinfected.

The dose and route of administration followed the New Zealand label directions for tylosin in lactating cows. Tylosin is a member of the macrolide antibiotic family that acts by inhibiting RNA-dependent protein synthesis by blocking translation at the ribosome. The macrolide family are generally effective against Gram-positive cocci (both *Staphylococcus* and *Streptococcus* spp.), moderately effective against *enterococcus* spp. and have a poor action against Gram-negative bacteria (Salmon et al 1998). Tylosin is a weak base, and therefore reaches higher concentrations in milk than in serum due to the low pH of milk compared to serum (Ziv 1980). However, at the time heifers were treated in the current study, little milk is present within the gland. Hence it is likely that the pH of the secretion from the mammary gland is similar to serum so that the concentration of tylosin was unlikely to have reached the same concentrations as achieved in the lactating gland.

The most commonly isolated species pre-calving was coagulase-negative *Staphylococcus*, which can be resistant to some antibiotics (Hodges et al 1984; Salmon et al 1998). Salmon et al., (1998) reported that erythromycin (another macrolide) was effective against *Staphylococcus* and *Streptococcus* spp. isolated from the mammary glands of New Zealand and Danish dairy heifers. However, MIC_{90}'s for these species ranged from 0.13 to 64 ug/ml, suggesting that some isolates were resistant.

It has been hypothesised that the presence of an IMI pre-calving is an indicator that the teat canal is open, due to either the absence of a teat plug, or the poor quality of a teat plug (Compton et al, unpublished). In multiparous cows, the absence of a functional teat plug increases the risk of new IMI (Williamson et al 1995). If this is the case then it is plausible that the tylosin may have cured existing infections but that reinfection occurred due to an open teat canal. However, analysis of this same data set at gland level failed to demonstrate a higher cure rate in tylosin compared to untreated glands (Parker et al., unpublished, chapter 4).

The use of antibiotics designed for lactating cows in heifers pre-calving, is 'off label' usage. These treatments may increase the risk of milk antibiotic residues in early lactation. However, only 3% of quarters treated with a penicillin and dihydrostreptomycin dry cow therapy product 3 months before calving had detectable antibiotic residues within 3 days of calving (Trinidad et al 1990b). Oliver et al., (1992) found that 17% of composite quarter milk samples tested positive on the day of calving for antibiotic residues in heifers treated with lactating cow cloxacillin approximately 7 days pre-calving (0% following that), but that 85 %, 28% and 0% of glands were positive at 0, 3 and 10 days post-calving, respectively, following cephapirin treatment. One further study (Owens et al 1994) reported no antibiotic residues detected at 5 days post-calving following cephapirin dry cow therapy 10-14 weeks earlier. Antibiotic residues were not measured in this study, so the risk of antibiotic residues with an injectable antibiotic cannot be described.

The risk that a heifer was removed ("culled") was unaffected by treatment in the current study. Compton et al (unpublished) found that the isolation of a major pathogen post-calving significantly increased the risk of culling in heifers drawn from the same farms as the current study. However, the herd-owners reasons for removal of these animals were due to a higher non-pregnant rate in animals with clinical mastitis than those without. The effect of treatment on the outcome culling may not have been significant in the current study due to the large number of clinical mastitis cases from which no bacteria were isolated from. Farmers may make some of their culling decisions based on the diagnosis of clinical mastitis in a heifer not on the actual outcome in that heifer. There was insufficient statistical power in the current study to analyse the specific reasons for culling.

High individual cow SCCs and/or clinical mastitis in heifers results in lower production during heifer's first lactation (De Vliegher et al 2005) and long-term production loss (Woolford et al 1983; 1984). However, in the current study infusion with a teat sealant or treatment with tylosin before calving had no effect on production in the subsequent lactation. However milk yield was determined only 4 times across lactation as this is common practice under New Zealand management systems. This relatively infrequent assessment of milk yield may have limited the ability to detect small changes in milk production between the treatment groups, especially where the potential loss associated with a disease process was short term and occurred in early lactation before the first herd test. Other confounders such as nutrition, climate, and milking management that resulted in new IMI during lactation independent of pre-calving treatment may also have increased variability in production, reducing the chance of detecting treatment differences.

While beyond the scope of the current study, it is likely that infusion of a teat sealant is economic, especially in herds with a high incidence of new IMI and clinical mastitis in their heifers peripartum. Mastitis imposes significant costs to the dairy producers and the industry (Wells et al 1998; Bradley 2002; Seegers et al 2003). Direct and quantifiable financial losses occur due to the cost of treatment and prevention, loss of cows due to death and premature culling from the herd, loss of milk for supply during and after treatment with antibiotics, and loss of milk production associated with elevated somatic cell counts. Intangible losses such as labour costs of treating cows, the increased risk of an inhibitory substance grade while treating cows with antibiotics

and the increased risk of other diseases are likely to occur. Losses associated with clinical mastitis range from US $100 to $400 per cow per lactation (Schepers and Dijkhuizen 1991; Kossaibati 2000). For a group of 100 heifers in which the clinical mastitis incidence was reduced from 20% to 15% following infusion of teat sealant pre-calving, the costs of treatment would be approximately $NZ800 (i.e. 100 heifers x 4 tubes teat sealant x $2/tube) while the saving would be 5 cases of clinical mastitis at $200 each or a total benefit of $1000.

Infusion of teat sealant, which has no antibacterial properties, into the mammary gland may increase the risk of iatrogenic infection via direct introduction of pathogens. It is also possible that the sealant itself may act as a nidus for infection if contamination occurs during infusion or due to increased risk of infection secondary to iatrogenic trauma to the teat end or teat canal. No documented cases of clinical mastitis pre-calving occurred associated with treatment, nor was there an increase in risk of IMI or clinical mastitis post-calving in the current study suggesting the above mentioned risks are manageable. However, in the current study technicians trained in aseptic technique undertook all procedures. Hence herd-owners adopting this technology need to be trained and made aware of the risks associated with it. Additionally, herd-owners need to be warned that flecks of the teat sealant may be present for some days post-calving and these cases do not need to be treated as clinical mastitis.

Conclusions

The findings of the current study demonstrated that teat sealant reduces the prevalence of post-calving IMI and incidence of clinical mastitis in heifers. This presents a novel, cost effective and practical option for farmers wishing to reduce mastitis in their heifers post-calving. The absence of interactions with the known covariates such as breed, herd, udder oedema and body condition score suggest that teat sealant is likely to be effective over a wide range of herd types and heifers.

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Table 16. Number of heifers treated (n), calving date, breed, prevalence of intramammary infection (IMI) pre and post calving, cumulative incidence of clinical mastitis (%) in the first 2 weeks post-calving, milk solids production (fat + protein kg/cow/d) and geometric mean SCC at each of the herd tests for heifers treated with infusion of teat sealant into all 4 glands (Teat sealant), treatment with 3 X 5g of parenteral tylosin (Tylosin), both infusion of a teat sealant and tylosin treatment (Teat sealant and tylosin) or with nothing (Control) ~ 39 days before calving.

		Teat sealant	Tylosin	Teat sealant and Tylosin	Control	Total
Heifers (n)		247	247	247	243	984
Lost to follow up (n)		21	21	19	22	83
Friesians (n)		90	103	90	91	374
Jerseys (n)		82	67	72	84	305
Crossbreds (n)		75	77	85	68	305
Culls/premature removals (n)		38	43	28	25	134
Mean Calving Date		26-Jul-04	27-Jul-04	27-Jul-04	27-Jul-04	27-Jul-04
Mean interval treatment to calving (days)		40.9	41.4	41.9	41.5	41.4
Mean interval calving to sampling (days)		1.99	1.98	2.00	1.97	1.98
Mean minimum teat height off ground (cm)		51.9	51.8	51.7	51.8	51.8
Mean body condition score pre-calving		5.9	5.9	5.9	5.9	5.9
Mean body condition score post-calving		5.0	5.1	5.0	5.0	5.1
Number with oedema post-calving (n)		123	141	135	158	557
Mean hygiene score post-calving		1.9	2.0	2.0	2.0	2.0
HT 1	Geometric mean SCC (X 1000)	47.7	45.4	43.5	52.3	47.1
	Milk yield (kg milk fat+protein/day)	1.31	1.33	1.32	1.32	1.32
	Mean herd test to calving interval	51	51	49	49	50
HT 2	Geometric mean SCC (X 1000)	46.7	49.9	43.1	45.3	1.66
	Milk yield (kg milk fat+protein/day)	1.21	1.20	1.21	1.23	1.21
	Mean herd test to calving interval	109	109	107	107	108
HT 3	Geometric mean SCC (X 1000)	47.0	54.9	47.3	52.7	50.4
	Milk yield (kg milk fat+protein/day)	1.09	1.07	1.09	1.08	1.08
	Mean herd test to calving interval	174	176	173	174	174
HT 4	Geometric mean SCC (X 1000)	92.5	99.2	93.4	100.3	96.2
	Milk yield (kg milk fat+protein/day)	0.72	0.71	0.72	0.72	0.72
	Mean herd test to calving interval	236	237	235	235	236
Pre-calving IMI1 n (%)		78 (31.6)	89 (36.0)	86 (34.8)	105 (43.2)	358 (36.4)
Post-calving IMI[1] n (%)		45 (18.2)	112 (45.3)	56 (22.7)	118 (48.6)	331 (33.6)
Clinical mastitis n[1] (%)		37 (15.0)	49 (19.8)	39 (15.8)	52 (21.4)	177 (18.0)

[1] Intramammary infection

Table 17. Number of quarters (n) per heifer diagnosed with clinical mastitis and of those the number and percentage of quarters per heifer that isolated no bacteria for heifers treated with infusion of teat sealant into all 4 glands (Teat sealant), treatment with 3 X 5g of parenteral tylosin (Tylosin), both infusion of a teat sealant and tylosin treatment (Teat sealant and tylosin) or with nothing (Control) ~ 39 days before calving.

		Number of clinical quarters				
		1	2	3	4	Total
Teat sealant	Clinical n	23	10	0	4	37
	No growth n (%)[1]	17 (45.9)	7 (18.9)	2 (5.4)[2]	2 (5.4)	28
Tylosin	Clinical n	38	9	1	0	48
	No growth n (%)[1]	6 (12.5)	2 (4.2)	0	0	8
Teat sealant and Tylosin	Clinical n	20	12	2	3	37
	No growth n (%)[1]	10 (27.0)	9 (24.3)	1 (2.7)	3 (8.1)	23
Control	Clinical n	43	4	2	3	52
	No growth n (%)[1]	15 (28.8)	0	0	0	15
Total	Clinical n	124	35	5	10	174
	No growth n (%)[1]	48 (38.7)	18 (51.4)	3 (0.6)	5 (0.5)	74 (42.5)

[1]Number of heifers with no bacteria isolated from clinical mastitis/number of heifers with clinical mastitis

[2]The 2 heifers with 3 quarters with clinical mastitis that isolated no bacteria, actually had 4 quarters with clinical mastitis

Table 18. Relative risk (RR) and 95% confidence intervals (CI) of post-calving intramammary infection (IMI) for heifers treated approximately 39 days pre-calving with nothing (Control; 243 heifers), infusion of teat sealant in all four quarters (teat sealant; 246 heifers) or injection of 5 g of tylosin on 3 occasions at 24 h intervals (tylosin; 246 heifers) or with both infusion of teat sealant in all four quarters and injection of 5 g of tylosin on 3 occasions at 24 h intervals (teat sealant and tylosin; 246 heifers) calculated from the multivariate analysis.

					95% CI		
Effect	Estimate	Standard Error	t Value	RR	Lower	Upper	P value
Intercept	-0.08	0.07	-1.18	0.92			0.25
Teat sealant	-0.85	0.10	-8.88	0.43	0.35	0.52	<0.001
Tylosin	0.02	0.06	0.28	1.02	0.90	1.15	0.78
Pre-calving IMI (358 heifers)	0.46	0.07	6.2	1.58	1.37	1.83	<0.001
Calving to sampling interval[1]	-0.22	0.03	-7.11	0.81	0.76	0.85	<0.001

[1]Interval between calving and sampling (0-5 days)

Table 19. Relative risk (RR) and 95% confidence intervals (CI) of clinical mastitis diagnosis for heifers treated approximately 39 days pre-calving with nothing (Control; 243 heifers), infusion of teat sealant in all four quarters (teat sealant; 246 heifers) or injection of 5 g of tylosin on 3 occasions at 24 h intervals (tylosin; 246 heifers) or with both infusion of teat sealant in all four quarters and injection of 5 g of tylosin on 3 occasions at 24 h intervals (teat sealant and tylosin; 246 heifers) calculated from the multivariate analysis.

					95% CI		
Effect	Estimate	Standard Error	t Value	RR	Lower	Upper	P value
Intercept	0.31	0.80	0.38				0.71
Teat sealant	-0.29	0.13	-2.31	0.75	0.58	0.96	0.02
Tylosin	-0.06	0.13	-0.52	0.94	0.73	1.20	0.61
Minimum teat height (cm)	-0.05	0.01	-3.04	0.96	0.93	0.98	0.002
Calving to sampling interval[1]	0.01	0.00	3.05	1.01	1.00	1.02	0.002

[1]Interval between calving and sampling (0-5 days)

Table 20. Factors affected somatic cell counts (SCC) in heifers following treatment approximately 39 days pre-calving with nothing (Control; 243 heifers), infusion of teat sealant in all four quarters (teat sealant; 246 heifers) or injection of 5 g of tylosin on 3 occasions at 24 h intervals (tylosin; 246 heifers) or with both infusion of teat sealant in all four quarters and injection of 5 g of tylosin on 3 occasions at 24 h intervals (teat sealant and tylosin; 246 heifers) calculated from the multivariate analysis.

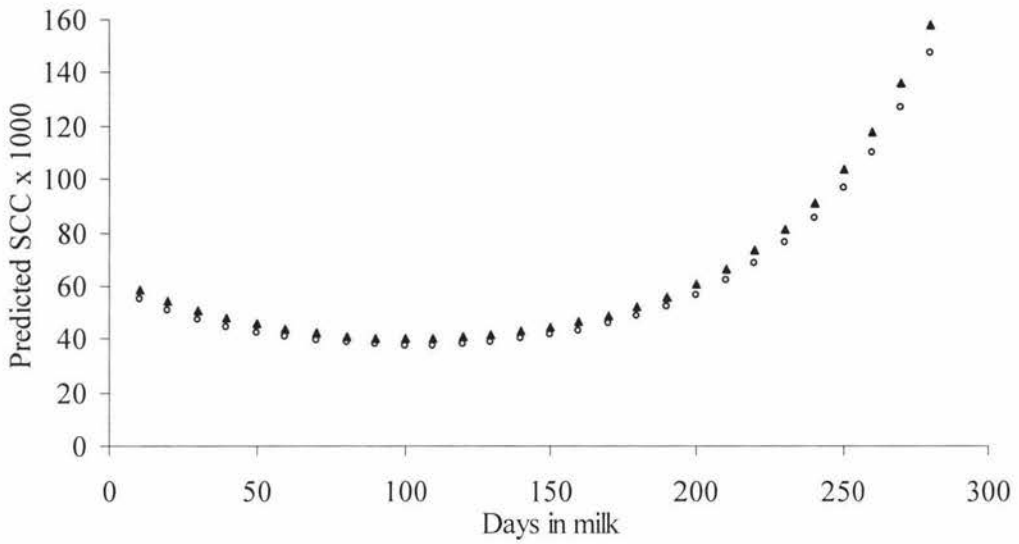
Effect		Estimate	Standard Error	t Value	Change in SCC[3]	P value
Intercept		1.81	0.03	54.22		<0.001
Teat sealant		-0.03	0.02	-1.74	1070	0.08
Tylosin		-0.02	0.02	-0.99	1039	0.32
Breed	Friesian (374 heifers)	0.09	0.03	3.36	1236	<0.001
	Jersey (305 heifers)	0.02	0.03	0.78	1050	0.43
	Crossbred[1] (305 heifers)	0				
Herd test to calving interval (days)		-0.00392	0.0003	-12.84	1009	<0.001
Herd test to calving interval[2] (days)[2]		0.00002	0.0000	Inf	1000	<0.001

[1]Reference category

[2]Herd test to calving interval squared

[3]SCC for reference category=64062 cells/ml

Figure 13. Predicted somatic cell counts (SCC) from the final model in heifers throughout lactation following treatment approximately 39 days pre-calving with infusion of teat sealant in all four quarters within heifers (▲) or no treatment with teat sealant (○).



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Chapter 7 Discussion

As well as testing some important hypotheses the studies in this thesis have posed further questions about the epidemiology and control of heifer mastitis. Risk factors for heifer mastitis can occur at the quarter, heifer and herd level. Intervention strategies may therefore need to occur at some or all of these levels to reduce heifer mastitis. This thesis identified some herd level risk factors for heifer mastitis and investigated the effect of a preventive approach at quarter level. However, the use of teat sealant, despite its preventive action occurring at the quarter level, would be implemented at herd level.

Heifer mastitis is a problem in New Zealand based on the findings in these studies and also the concurrent studies carried out by Chris Compton. The herd level risk factors identified as being associated with a higher incidence of heifer mastitis included increasing stocking rate, managing the lactating cows in only one group, increasing the number of cows for each person milking, increasing the incidence of clinical mastitis in cows and increasing the per cow production. A number of significant risk factors were identified at the bivariate analysis that did not remain in the final multivariate model. More research is needed to determine if some of these factors are genuinely important. Additionally the mechanisms by which these significant factors are affecting the risk of heifer mastitis need to be identified

The two intervention studies (and work of Chris Compton's) identified that the prevalence of pre-calving IMI in heifers in New Zealand is lower than in previous studies undertaken in the Northern Hemisphere. The lower prevalence may be associated with the management systems in New Zealand and further evaluation of the interaction of management with pre-calving IMI may provide strategies to decrease the risk of pre-calving IMI. In the current studies, pre-calving IMI was a risk for IMI post-calving, whether this is because there is something specific about those quarters that make them higher risk (e.g. absence of a teat plug), or something about that heifer that increases her risk of subsequent IMI, is yet to be elucidated. Identifying whether heifers have a functional teat plug pre-calving and what the risk factors are for presence/absence of a teat plug and functionality of the plug may be important data required to understand the epidemiology of pre and post-calving IMI.

Intramammary infection has been identified in an American study in unbred dairy heifers, which suggests pre-calving IMI can occur well before the mammary gland has developed. However, in pasture-based systems there is no data about the age at which heifers acquire IMI. Identifying at what stage, and how these pathogens invade the teat canal will be an important finding. It has been suggested that spread by flies is a likely cause in the southern states of America. However, in New Zealand the species of flies are different from the biting species of the southern USA, were if flies were to play a role in New Zealand the transmission of bacteria would be purely by mechanical means. If IMI is occurring via the teat canal earlier in the heifer's life, it may be hypothesised that earlier administration of a teat sealant may be more beneficial. This may be occurring through cross suckling from other heifers or from contamination of the teat end via other means. The alternative hypothesis for the route

of pre-calving IMI is haematogenous, possibly from colonisation of bacteria in the gastrointestinal tract following feeding of mastitic milk as calves.

Infusion of a teat sealant pre-calving, prior to the high-risk period for new IMI, is a novel and practical option for farmers wishing to reduce the prevalence of IMI and clinical mastitis in heifers post-calving. Despite the fact that pre-calving IMI was a risk for post-calving IMI, the incidence of new IMI occurred just prior to calving (Compton et al). Physically preventing IMI by infusion of a non-antibiotic teat sealant was shown to be an effective option.

Further research is required to understand more about what the specific risk factors are at herd, heifer and quarter level for new IMI in the week before calving, to aid in the development of preventive strategies for heifer mastitis. Other prevention specific strategies that could be evaluated include milking heifers pre-calving, changing management and feeding practices pre-calving or the repeated application of teat disinfectants pre-calving.

The use of injectable antibiotics pre-calving did not prove effective in the studies in this thesis. The reasons for the failure of parenteral therapy to cure existing infections need further investigation. A failure to achieve concentrations above MIC appears to be the most likely explanation. Due to the pKa of tylosin and the lower pH of milk, the concentrations of tylosin achieved in the mammary gland are much greater than those in serum. However, in the non-lactating gland (heifers ~ 39 days pre-calving) the pH is more likely to approximate that of serum and therefore the concentrations of tylosin in the gland are unlikely to be effective against IMI. Whether other active compounds with different pharmacokinetics, or the use of tylosin closer to parturition, would be more effective require further study. The use of intramammary antibiotics in heifers pre-calving has proven effective at decreasing IMI post-calving, clinical mastitis and SCC. However, there is and associated increased risk inhibitory substance grades in the milk following antibiotic therapy pre-calving. It is preferable if prophylactic use of antibiotics is kept to a minimum in the food production industries.

It is important that further research into heifer mastitis is funded by the dairy industry worldwide in attempt to create adequate recommendations for prevention for dairy farmers. The true cost heifer mastitis to the herd-owner and to the dairy industry is unknown in New Zealand. Further research is also needed to identify the cost benefits of reduction in heifer mastitis at both the individual herd and industry level.

Appendix 1 Heifer survey questionnaire

1. What is your effective farm area (Ha)? _____
2. What type of milking parlour do you milk the cows in?

rotary ☐ herringbone swing over ☐ herringbone double up ☐ walk thru ☐

3. How many sets of cups do you have in the shed? _____
4. How many cows are you going to calve this season? _____
5. How many heifers are you going to calve this season? _____
6. What is the planned start of calving for the main herd? _____And the heifers? _____
7. What was your total production (kgMS) for last season 2002/2003? _____
8. Do you run a springer mob? Yes ☐ No ☐

If so when do you draft cows into the mob?

>2weeks before calving ☐1-2 weeks before calving ☐ <1 week before calving☐ other☐

9. Do you run a colostrum mob? Yes ☐No ☐
10. Do you run multiple milking herds? Yes ☐ No ☐ If so how are they split?

age ☐ body condition ☐ calving date ☐ other ☐

11. Do you run multiple dry cow mobs? Yes ☐ No ☐ If so how are they split?

age ☐ body condition ☐ calving date ☐ other ☐

12. When is the calf removed from the cow (time of day)? _____

13. When is the cow first milked (time of day)? _____
14. Do you stand springers off pasture if there is heavy rain? Yes ☐ No ☐
15. Where do you get your replacements? bred at home ☐ purchased ☐
16. If you **purchased** heifers, at what age were they purchased?

<9 months ☐ 9-15 months ☐ 15-24 months ☐ older ☐

17. If you **reared** your own replacements were they fed (tick one or more):

milk replacer ☐ whole milk ☐ antibiotic milk ☐ colostrum ☐

18. Between weaning and mating (i.e. 3-12 months) were the calves

at home ☐ at a runoff ☐

19. If the calves were at home did they follow the cows grazing? Yes ☐ No ☐
20. From 12 months of age were the heifers

at home ☐ at your runoff ☐ at a graziers ☐

21. Are the heifers run through the shed before calving? Yes ☐ No ☐

a) If **yes,** how many times before calving? _____

b) Do you teat spray them at this time? Yes ☐ No ☐

c) Do you check for mastitis at this time? Yes ☐ No ☐

d) What other procedures do you do when the heifers go through the shed?

_____

22. Do you tail dock heifers? Yes ☐ No ☐

a) If **yes** do you do it pre-calving ☐ during lactation ☐ post calving ☐

23. Where are the heifers run before calving?

With the main herd from June 1 ☐ Join main herd after calving ☐

Join main mob at another time (date)? _____

24. Is oxytocin (let down hormone) given to every heifer? Yes ☐ No ☐

25. Is oxytocin given if heifers don't let down their milk? Yes ☐ No ☐

26. Are any other let down methods used (what)? _____

27. Do you use teat spray or dip? (pre ☐, post ☐, pre and post ☐)

b) If so, what do you use?

Iodine (yellow) ☐ Chlorhexidine (pink or blue)☐ other ☐

28. Do you antibiotic treat cows if they are **only** positive on RMT, litmus, conductivity meter tests or with high cell count from herd test (i.e. **no** visible change in the milk or gland)? Yes ☐ No ☐

29. Do you strip the colostrum cows to check for mastitis:

at the first milking ☐ once daily ☐ twice daily ☐

first and last milking in colostrum mob ☐ other ☐

30. How many people do you have milking at a time? 1 ☐ 2 ☐ 3 ☐

31. Do you think heifer mastitis is a problem in your herd? Yes ☐ No ☐

Any other comments on heifer mastitis would be greatly appreciated below. Thank you for your time.

_____

_____

_____

_____

_____

_____

_____

_____

_____

To make the recording of clinical cases easier for you we can provide you with the recording sheets on adhesive paper to stick into your yellow book.

Would you like a sheet of 4 recording sticky labels? Yes ☐No ☐

Clinical Mastitis Recording Sheet 03/04

Date	Cow #	Quarter	Heifer (Yes/No)

Appendix 2

Distribution by herd of proportion of heifers with clinical mastitis.