Mastitis in two experimental herds: A case study in organic and conventional dairy systems

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1. ABSTRACT

Mastitis costs amount to about 11% of the productive capacity of the dairy industry. In New Zealand, practices for its control have been adapted to constitute the SAMM plan (Seasonal approach for managing mastitis), with a selective use of Dry cow therapy (DCT, a long lasting antibiotic) based on individual somatic cell counts (SCC) and clinical cases records from the previous lactation.

Nowadays, restricted use of antibiotics constitutes an important factor in dairy production. Organic production methods, which constrain the use of conventional medicines and treatments, are consequently increasing in importance.

Research in conventional herds has shown the prevalence of different pathogens in New Zealand, highlighting the importance of *Streptococcus uberis* around calving and of Coagulase-negative staphylococcus (CNS) and *Staphylococcus aureus* later in lactation. However, there is no comparable data coming from organic herds.

The present study assessed the mastitis infection status of two farmlets located at the Dairy Cattle Research Unit of Massey University. Single quarter milk samples from 95 cows, 45 under organic production (first and second year of organic certification) and 50 under a conventional system were taken on four occasions: mid-lactation (frozen); prior to dry-off (season 2003-04); after calving and 14 days post-calving (season 2004-05). Milk samples were cultured for bacterial analysis. SCC from monthly herd tests, and clinical mastitis cases were recorded and analyzed.

Significant differences between herds were shown only for growth of *Staphylococcus aureus* in all four periods, and for cows positive for *Streptococcus* spp. 14 days post-calving, with a higher percentage of infections in the organic herd in all cases.

Somatic cell counts data from individual cows in season 03-04 showed mean values of 116 and 102 (thousand cells/ml) for the organic and conventional herd respectively, with no significant differences between them, not during the first half of season 04-05, where the mean values were 91 and 67 (thousand cells/ml).

No differences were shown in clinical mastitis cases between herds. However a slightly higher frequency of cases was present in the organic herd (31%) compared to the conventional herd (28%) for the season 03-04. The frequency of clinical cases for the first half of season 04-05 was 16% for the organic herd and 17% for the conventional herd. Further studies in these two herds should be performed to analyze the effect of *Staphylococcus aureus* over time, especially in the organic herd.

Results suggest that infections caused by *Staphylococcus aureus* in organic herds could spread due to the prohibition of antibiotics. Control of this contagious pathogen is important in all herds, but especially in organic conditions, where further preventive and control methods should be established to avoid increasing infections.

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4. INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland resulting from infection with a pathogenic microorganism (Sears and McCarthy, 2003a).

It affects almost all mammalian species, but for dairy animals it represents the most costly disease that they are subjected to as a consequence of the decrease in milk yield it causes and the need for treatments that lead to the discard of milk contaminated with antibiotics. Expenses related to veterinary fees, extra labor and treatments, add up to about 10% of the productive capacity of the dairy industry (Weimer, 1998). In the New Zealand context, during the 1991-92 season losses were estimated of NZ$14,000 per herd, when an average bulk tank somatic count of 400,000 cells/ml was assumed, considering different sources of loss, as shown in Table 4.1 (Holdaway, 1992a).

Table 4.1 Estimated losses for the producer as a consequence of mastitis (91-92)

Source of loss	Cost to the producer
Loss of milk production	$ 9,905.00
Labor related to clinical mastitis	$ 163.86
Antibiotics used in clinical mastitis	$ 216.51
Discarded milk	$ 62.68
Labor related to dry cow therapy	$ 27.60
Dry cow therapy antibiotics	$ 459.48
Culling	$ 4,614.00
Total cost	$ 14,639.13

Therefore, due to high costs caused to the producer, its treatment and control are an important issue in any dairy farm. Mastitis control programs include the use of antibiotics to treat infections during lactation, when short term antibiotics are used. However, for better results, dry cow therapy is a recommended treatment to eliminate existing infections at the end of the lactation and to prevent new ones during the dry period. This treatment consists of infusing a long-lasting antibiotic into the quarter at the end of the lactation. Different strategies can be used when applying dry cow therapy, where some producers will treat all the quarters of all cows and others will treat just the infected cows or quarters (selective therapy). In New Zealand, 81% of the 2,460

participants from a survey in 1986 affirmed the use of selective dry cow therapy in "infected" cows based on individual somatic cell counts (SCC) and clinical mastitis records during lactation (Laycock *et al.*, 1987).

In recent years, alternative methods of treatment such as homeopathy have been tested and organic dairy production has increased around the globe. The rules of organic systems do not allow the use of prophylactic medication and if drugs must be used, which is considered extraordinary, the quarantine and milk discard period is very long, or removal of the treated animals from the herd is required. Therefore, mastitis acquires a unique position and importance when no antibiotic treatments can be used.

Normally, in order to control and treat mastitis a herd history, isolation, identification and susceptibility of the pathogen involved are required (Quinn *et al.*, 1994). Consequently, since organic dairy production has been continuously growing, the study and understanding of mastitis patterns in organic systems has increased in importance in recent years.

In New Zealand conditions, mastitis is managed on a seasonal basis and most of the clinical cases occur in the period around calving in spring time. The present study analyzed data from two herds: one managed conventionally and the other in its first year as a fully certified organic system, from mid lactation until the early stages of the following lactation. The aim was to provide a detailed and comprehensive picture of the mastitis status of cows in the two herds over this critical period.

4.1. Importance of Mastitis

Bacterial infection is considered to be the main cause of inflammation of the mammary gland and about 130 microorganisms have been isolated from mastitic milk samples, but Staphylococci, Streptococci and *Enterobacteriaceae* constitute the most common pathogenic infection agents (Quinn *et al.*, 1994). The udder is constantly exposed to many different pathogens, and exposure to a high bacterial load and susceptibility to infection can result in a new intramammary infection (IMI). Susceptibility depends on many factors, but infection is mainly due to the milking process.

Each quarter of the mammary gland comprises a teat with a streak canal and a cistern, a gland cistern, milk ducts and secretory glandular tissue (see Figure 4.1). The latter is formed by millions of minuscule sacs known as alveoli that are surrounded by the epithelial cells that synthesize milk, and by muscle cells that cause milk ejection. Blood vessels provide the alveoli with nutrients that are synthesized into milk, which accumulates in the alveolar spaces, milk ducts and cisterns. During milking about 90% of the total fluid accumulated is removed from the ducts (Bramley *et al.*, 2003).

When mastitis occurs, pathogens enter the gland through the teat canal, colonizing the duct system and alveoli, causing an inflammatory reaction (Oliver and Sordillo, 1988). These pathogens could come from the environment (e.g. *Escherichia coli*) or from the udder of another animal (e.g. *Staphylococcus aureus*) via the milking machine or the milker's hands.

Table 4.2 Changes in Milk Composition related to a high SCC

Constituent	Normal Milk (%)	High SCC Milk (%)	% of normal milk
Solids-non fat	8.90	8.80	99%
Fat	3.50	3.20	91%
Lactose	4.90	4.40	90%
Total Protein	3.61	3.56	99%
Total Casein	2.80	2.30	82%
Whey protein	0.80	1.30	163%
Serum Albumin	0.02	0.07	350%
Lactoferrin	0.02	0.10	500%
Immunoglobulins	0.10	0.60	600%
Sodium	0.05	0.10	184%
Chloride	0.09	0.14	162%
Potassium	0.17	0.15	91%
Calcium	0.12	0.04	33%

(Hogan *et al.*, 1999)

As a consequence of mastitis, milk characteristics change during the infection due to the presence of bacterial by-products, which affect milk composition. Fat content may decrease to less than 3%. In addition, chloride is increased 1.5 times and lactose decreases around five fold since pathogens use it as growth substrate. Total protein content does not change dramatically but its composition is affected, with a decrease in casein and an increase in whey protein (Weimer, 1998). Table 4.2 shows some of the compositional changes found that are typical of many studies (Hogan *et al.*, 1999).

Therefore, infected quarters produce lower yields of milk with a lower content of fat, lactose and casein than healthy quarters. Moreover, even after elimination of infection, milk yield does not always recover completely, sometimes even during the following lactation. Effect of infection during the dry period on milk yield has been measured and results showed that secretory tissue damaged by infections is not repaired completely after the dry period (Smith *et al.*, 1968).

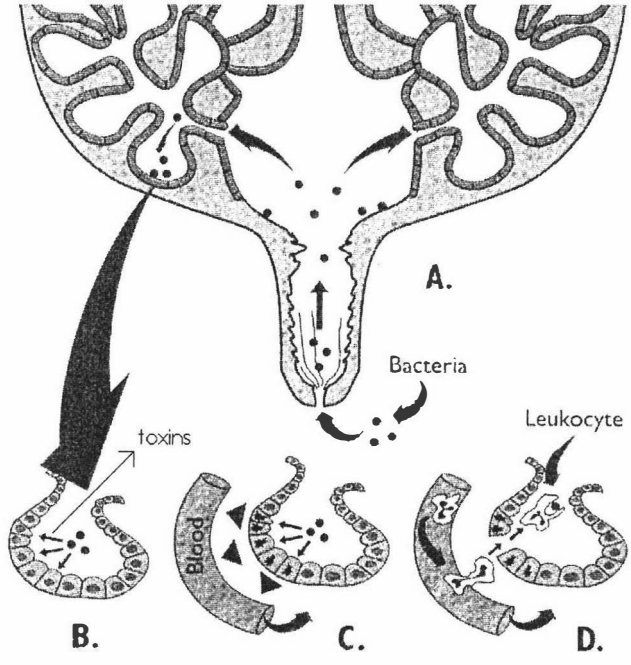
Furthermore, improvement in milk yield as a consequence of breeding programs orientated towards a higher milk production has been shown to be associated with an increase in the prevalence of mastitis due to the genetic correlation between milk yield and mastitis, from -0.3 to +0.66 (Zecconi and Smith, 2003), possibly because higher milk yields are associated with faster milk flow rates through more patent teat canals.

Clinical mastitis is usually related to signs that can be detected by sight or touch, such as abnormal milk, swollen, hard, hot glands and sometimes illness of the animal. On the other hand, subclinical mastitis generally presents a visibly normal udder and milk, but milk with high somatic cell counts (SCC) due to the presence of a larger number of leukocytes (Sears and McCarthy, 2003a). The latter can be detected by laboratory methods to count the somatic cells in milk, with subclinical mastitis indicated by counts above 5×10^5 cells/ ml (Zecconi and Smith, 2003).

If the bacteria that causes the infection is obtained from another animal (mainly during the milking), mastitis is classified as contagious (e.g. *Staphylococcus aureus* and *Streptococcus agalactiae*). On the other hand, if it is obtained from the environment, it is called environmental (e.g. *Streptococcus uberis* and *Escherichia coli*)

Infection can take place via the blood stream or by trauma to the udder. However, the teat canal is the usual route through which it occurs (Figure 4.1 A). Even though the features of the canal provide considerable defense against infections, this defense weakens in older or high-yielding cows. Infection, regardless of the means of entry to the gland, results in different responses including phagocytosis of invading pathogens by polymorphonuclear neutrophils (Figure 4.1 D), production of antibodies to resist bacterial adherence and neutralization of toxins (Weimer, 1998).

Figure 4.1 Scheme of an intramammary infection



(Hogan *et al.*, 1999)

During the last few decades the main bacteria causing mastitis has been *Staphylococcus aureus,* which has overtaken *Streptococcus agalactiae* as the most important species (Weimer, 1998).

Many methods have been used to determine economical losses due to mastitis; however, none of them is accurate since it is difficult to determine how much milk the cow would have produced if it had been free of infection. Nevertheless, techniques such as between-herd yield comparisons; between-cow comparisons; within-udder yield comparisons; within-cow yield comparisons and studies between identical twins have been used in attempts to obtain the closest estimate possible (DeGraves and Fetrow, 1993).

Losses have been attributed mainly to a decrease in milk production from subclinical mastitis, with a range calculated from 10 to 26%. On average, milk production is reduced by 190 kg per unit of increment in a linear somatic cell count score, a logarithmic scale of SCC (DeGraves and Fetrow, 1993). In the case of clinical mastitis, research has shown that losses are higher if infection presents before the peak of

lactation, causing a reduction of 11% at this stage, compared to 6.4% later in lactation (DeGraves and Fetrow, 1993).

Results from a study with identical twins in New Zealand showed that, for a herd infected with *S. aureus* with a bulk tank SCC of 739,000 cell/ml compared with an infection-free herd of 66,000 cells/ml, the heifers from the infected herd were producing 7.8% less than the heifers in the clean herd. However, older cows showed a reduction of just 1.7% compared with the clean herd, since the uninfected quarters of these cows showed an increment in milk yield that compensated the decrement of production in the infected quarters (Woolford *et al*., 1983).

Losses in USA have been estimated around 185 USD per cow per year (Bramley *et al.*, 2003), while in New Zealand, in 1991, losses of 86 NZD per cow per year were estimated in herds averaging 400,000 SCC (Holdaway, 1992b).

4.2. The New Zealand Scenario

Dairy production in New Zealand is based on grazing and its objective is to synchronize the herd's feed demand with the farm's feed supply (pasture growth) in order to support milk production. To balance feed supply and demand, management tools including stocking rate, irrigation, supplements, crops, fertilizers, calving dates and dry-off dates are used. As a consequence, most animals calve in late winter-early spring and are dried-off in late summer in order to keep a cycle in the demand for feed that is similar to that shown by pasture production. These farm systems create a short (10-14 weeks) calving period of intense activity, where management and udder health acquire special importance.

Management during early lactation has shown to be very important since most of the infections appear during this period, especially those due to environmental pathogens such as *Streptococcus uberis* (Woolford and Lacy-Hulbert, 1996). In addition, since cows spend most of the time on fresh, clean pastures, infections caused by *Escherichia coli* and other enterobacteria and Gram-negative bacteria generally have a low incidence in New Zealand (Woolford and Lacy-Hulbert, 1996).

However, the last mastitis survey in New Zealand took place almost 40 years ago, where data from 130 herds with a total of 10,861 animals was analyzed (Brookbanks, 1966). Results showed that 27% of the animals were infected with *Staphylococcus aureus*; 13.7% with *Streptococcus agalactiae*; 0.5% *Streptococcus uberis*; 2.3% with *Streptococcus dysgalactiae* and just 0.6% showed different organisms such as *Escherichia Coli* and *Corynebacterium pyogenes.*

At present, mastitis control in New Zealand is managed on a seasonal basis focused on calving (in spring) and drying off (in autumn), where most of the infections through the dry period and at calving are due to *Streptococcus uberis* (Woolford, 1997). Recent trials have shown that Coagulase-negative staphylococci (CNS) and *Staphylococcus aureus* are increasing in importance through the rest of the lactation (Williamson et al., 1995, McDougall, 1998).

Somatic cell counts (SCC) of individual cows are obtained through regular herd tests around the country. In those, individual milk samples are collected once a month or every two months from every milking animal in the herd and milk volumes, milk fat, protein yield and somatic cell counts are provided. The latter are used as indicators for subclinical intramammary infections. In addition, somatic cell counts from the herd's bulk milk are available for every consignment. In 1975, when somatic cell counts began in New Zealand, not much importance was given to them, but with their help, by 1991 the average bulk tank somatic cell count around the country was slightly higher than 400,000 cells/ml, which is nowadays the maximum SCC limit in Europe (Woolford, 1997). This boosted the use of somatic cell counts as a tool for monitoring herds and controlling mastitis. In season 2003-04, 77% of the herds in New Zealand used herd testing and the SCC national average was of 220,000 cells/ml (LIC, 2004).

Furthermore, the plan called Seasonal Approach to Managing Mastitis (SAMM) has been established since 1993, based on five key productive periods in New Zealand's dairy production system: calving period (early spring); lactation; late lactation; drying-off (autumn) and dry period (winter). The SAMM plan follows the principles of the "five point plan" developed in the United Kingdom and extensively used in many countries, which for many years was used around New Zealand. The main elements of the five point plan are post-milking teat disinfection; total dry cow therapy for all cows; therapy of clinical cases during lactation; maintenance of milking machines and culling

of problem cows (Smith and Hogan, 2001), but with no reference to the detection or treatment of subclinical cases. However, under New Zealand conditions this plan was modified to include cows with high SCC (subclinical cases) and the use of Dry cow therapy on selected cows, where cows to be treated are chosen based on individual data from clinical and subclinical mastitis (high SCC) through the lactation (Laycock *et al.*, 1987).

Research done in the late 1980's and early 1990's in the Manawatu region showed that an average SCC higher than 120 000 cells/ml and 150 000 cells/ml in the case of heifers and multiparous cows respectively was a reliable indication of the presence of an intramammary infection (Holdaway, 1990) and those standards became then accepted under the SAMM plan.

As a consequence of a general lack of pre-milking wash and foremilk inspection in New Zealand herds, mastitis detection in many farms is done through observation of the udder and its quarters, checking for clots on the milk filter daily, monitoring SCC in the herd's bulk milk and herd test results for individual cows at intervals of two or three months (Laycock *et al.*, 1987). In most herds, teats are disinfected after every milking by spraying them with a sanitizer, as opposed to dipping the teats as in most other countries. However, many farms stop sanitizing teats in summer, after the high risk period of infections in early lactation is finished (Woolford, 1997).

The national SCC average is now about 220,000 cells/ml, but its seasonal fluctuation still represents an important issue to dairy producers with higher counts in early and late lactation, causing difficulties in compliance with the upper limit currently allowed by the industry of 400,000 cells/ml (Smith and Hogan, 2001).

The objective of the SAMM plan is to reduce the national average of SCC to 150,000 cells/ml (Joe, 1993). Future trends in mastitis management will fill the gaps in this plan, through the use of tools like monitoring milk conductivity or enhancement of immunologic properties of the mammary gland (Woolford and Lacy-Hulbert, 1996). In addition, consumers are demanding higher food safety, improved animal welfare and less indiscriminate use of antibiotics, which forces research to focus on developing mastitis control methods which use little or no antibiotics. Organic milk production systems are considered to be closely compatible with these general aims.

5. LITERATURE REVIEW

5.1. Dry period: A high risk phase for Mastitis

During the non-lactating period, the mammary glands go through a critical phase that will affect the upcoming lactation. In general, most of the development and growth of mammary tissue takes place at this time (Oliver and Sordillo, 1988).

Dairy cows are more susceptible to new intramammary infections (IMI) during the dry period, especially to those caused by environmental pathogens. This is particularly associated with an increment of internal pressure after milk removal is stopped at dry-off and when colostrum accumulates before calving, causing a shortening and dilation of the teat canal (Oliver and Sordillo, 1988).

The highest rates of infection appear during the early dry period, with lower rates in mid-dry period that increase again as calving gets closer. After dry-off, the udder is more susceptible to new infections since flushing of bacteria from the teat is terminated when milking ceases. In addition, dilation of the teat canal, caused by increased internal pressure, allows entrance of bacteria more easily (Figure 4.1), especially since the teats are no longer disinfected. Phagocyte function is also reduced since phagocytes start engulfing milk residues instead of bacteria (Bramley *et al.*, 2003).

As the dry period advances, the incidence of new intramammary infections decreases as udder pressure diminishes and leukocyte concentration increases, together with non-specific antimicrobial factors. Finally and most important, a keratin plug forms in the teat canal that protects the teat from the entrance of new bacteria. The period before teat closure varies widely and results from research in New Zealand, showed 45-55% of quarters still open seven days after dry-off and 5% open 60 days after dry-off (Williamson et al., 1995). However, as calving approaches, the risk of infection increases again due to an accumulation of fluids and colostral components in the gland, leakage of fluid through the streak canal and other factors (Bramley *et al.*, 2003).

Early research by Neave *et al.* (1950) studied infections during the dry period with a Shorthorn herd through a period of three years, by sampling animals seven days before dry-off, then one, two and three weeks after it. Further sampling took place one week before calving, and seven and ten days after parturition. Infected animals were sampled every week until the third week after calving. Results showed that 48% of the animals got infected during the dry period (representing 24% of the quarters). From these infections, half persisted through lactation and half of those persisting infections became clinical.

In addition, results showed that during the first three weeks after dry-off, the rate of persisting new infections was significantly higher (6.25 times) than during the previous lactation. This rate decreased in the last part of the dry period, with most of the new infections occurring during the first 21 days after calving, with 51 new infections from 58 in total (Neave *et al.*, 1950).

Moreover, most of the infections reported during the dry period were caused by aesculin positive streptococci (*S. uberis* and *Enterococcus*, environmental pathogens), and only a few by *Streptococcus agalactiae* and coliforms. However, rates of infection caused by *Streptococcus agalactiae* and *Staphylococcus aureus* (contagious pathogens) remained similar during both lactation and dry period. Considering the entire dry period (14 weeks), with 89 cows analyzed, rate of infections was 1.64 per week compared to 1.12 infections per week for the previous lactation (41 weeks) (Neave *et al.*, 1950).

Since then, the concept of the udder being more susceptible to infections at the beginning of the dry period has been extensively supported, since bacteria get into the teat canal more easily and it has been suggested that the higher resistance of the later phase of the dry period is mostly due to the keratin plug that prevents the entry of bacteria (Cousins *et al.*, 1980).

The latter has been supported by more recent research, which has shown that up to 95% of the new infections appearing in the dry period are caused by environmental pathogens and that 36% of these occur during the early part of the dry period (Dingwell *et al.*, 2003).

Likewise, a study was done with a herd of 160 cows free of *Streptococcus agalactiae* and with a low presence of *Staphylococcus aureus*, where milk samples were taken 14 and 7 days prior to dry-off; at the last milking of that lactation; at the day 7th and 14th of involution; 14 and 7 days before parturition and 7 and 14 days after it (Oliver, 1988). Major pathogens were present more frequently during the dry period and at calving than during the rest of lactation. To illustrate this, by late lactation 16.8% of quarters were infected with *C. bovis* and Coagulase-negative staphylococci whereas during early involution (early dry period) 23.3% of quarters were infected with major pathogens, mainly streptococci and coliforms. From the infections detected in the early dry-period, 69% were acquired after the milking had stopped and about 31% remained from late lactation (Oliver, 1988).

Results of a study over a period of five years in a herd with 220 animals agreed with these concepts, where 87% of quarter infections took place in the dry period or during the first seven days after calving (Hillerton *et al.*, 1995). In this trial, herd tests were taken at the beginning and end of the study in addition to quarter samples at calving, one week after parturition and before drying-off. Clinical cases were also sampled before treatment and two and three weeks after treatment.

Health status at drying-off was similar over the five year period, which suggests that persistent infections were present despite antibiotic treatments. However, a decrease in staphylococcal infections and an increase in streptococcal infections at drying off were detected at the end of the study. Despite a mastitis control plan, the incidence of clinical cases remained the same through the entire period (around 23-50 cases per 100 animals per year). Nevertheless, somatic cell count (SCC) was reduced from ~300,000 to 200,000 cells/ml (Hillerton *et al.*, 1995).

It was observed that most of the infections found at calving were new infections acquired during the dry period and close to parturition. Also, the rate of those infections was higher in older cows than in heifers, and the majority were first detected in the calving sample (Hillerton *et al.*, 1995).

Because of the agreement among many trials over the last 50 years, it is broadly considered that the first 15-21 days of the dry period represent the highest risk to new

infections appearance, and environmental pathogens play an important role during this phase.

5.2. Factors affecting new Intramammary infections

In general, it has been known since early research that older animals will present a higher rate of infection than younger animals (Neave *et al.*, 1950). In more recent studies it has been observed that cows after the fourth or greater lactation show higher rates of infection than younger animals (Dingwell et al., 2003). Basically, it is believed that this happens as a result of a more patent streak canal as the cow gets older due to loss of "muscle" tone in the streak canal, although the increased exposure to milking and milking machines in older cows must also be an important influence. Results from a trial in 1983 found that 2.6% of quarters from cows in their first or second lactations were infected at calving against a 23.8% of quarters from cows in their third or greater lactation (Oliver and Mitchell, 1983).

Another study showed that the percentage of infected quarters at calving was similar for all ages, with 3.1% for first lactation animals; 2.9% for cows in their second and third lactation and 3.8% for fourth or greater lactations. However, animals of a fourth or greater lactation presented a twofold higher infection rate during the first half of the dry period than during the second half of it (Todhunter *et al.*, 1995). In addition it is expected that older animals will present a higher infection rate by coliform organisms than young animals (Smith *et al.*, 1985).

During lactation, the main "source" of new infections is the milking process of the milking machine. Milking machines work on the basis of a vacuum created outside the teat which removes the milk through the streak canal. However, when excessive vacuum levels are present, damage to the teat canal occurs, that can lead to entrance of bacteria. Also, sudden changes in pressure could create reverse flow of milk leading to a cross contamination between quarters or cows. Finally, malfunctions of the machine or incorrect use of it could cause trauma to the teats or an incomplete milking, that could increase the exposure of the teats to bacteria or reduce the amount of bacteria and toxins removed at milking (Bramley *et al.*, 2003).

During the dry period, the occlusion of the teat canal by a keratin plug still represents the most important barrier against the entry of bacteria. A study in New Zealand documented the rate of closure by the streak canal at this time. After 30 to 40 days of the dry period, 50% of the teats were closed and at 60 days 95% were closed. However the data showed that 5% of the teat canals remained open during the entire dry period (Williamson *et al.*, 1995).

Likewise, it has been determined that from animals with a milk production of about 21 kg or higher at dry-off, 47% of quarters presented an open teat canal six weeks after dry-off against just a 19% of quarters from animals with a production lower than 21 kg (Dingwell *et al.*, 2003). In addition to that, as stated before, genetic susceptibility to mastitis is positively correlated to high-milk production; therefore, high producing animals are more susceptible to mastitis. However, just 10% of mastitis resistance is considered to be related to genetics and the rest is related to management (Bramley *et al.*, 2003).

5.3. Most common mastitis causing pathogens

5.3.1. Streptococcus uberis

This is a Gram positive, catalase negative cocci that hydrolyzes aesculin. *Streptococcus uberis* is considered the main cause of infection during the dry period and around calving. It is transmitted via the environment since this pathogen is usually a contaminant of the teat end. In grazing situations it appears as a contaminant when animals remain on the same area of pasture for prolonged periods (Hillerton and Berry, 2003).

However, even if *S. uberis* is considered an environmental pathogen, it could be transmitted from animal to animal or between quarters but with a very low incidence, since infections within a herd are caused by many different strains of bacteria. In New Zealand, *S. uberis* is considered the most important pathogen causing mastitis around the dry period (Williamson *et al.*, 1995).

5.3.2. Streptococcus agalactiae

This is a non-motile, Gram positive bacteria, with a diameter of 0.6-1.2 μm which usually forms long chains composed of pairs of cocci.

Streptococcus agalactiae, which is one of the most contagious species of streptococci, has decreased in importance as a pathogenic cause of mastitis due to improvements in hygiene at milking, management and new therapeutic methods (Hillerton and Berry, 2003).

Streptococcus agalactiae mainly causes contagious subclinical mastitis and as a consequence, losses in yield and milk quality. In New Zealand however, it has become one of the least important bacteria in clinical mastitis cases (Becker, 1994).

In 1944, Christie, Atkins and Munch-Petersen found a test response that identifies it from the rest of the streptococci. When grown next to a Staphylococcus producing an α toxin, a clear hemolytic zone appears between them, which also results positive for all Streptococci belonging to the Lancefield group B. This method, the CAMP test, is therefore used as a routine method for detecting *S. agalactiae* (Murphy *et al.*, 1952).

5.3.3. Staphylococcus aureus

This bacteria is a Gram-positive, catalase-positive, non-motile coccus, with a diameter of 0.5-1.5 μm, that grows in groups or aggregates that form up to three-dimensional clusters of cells (Asperger, 1994).

Its growth is most rapid in aerobic conditions, but it can grow in anaerobic conditions. Most strains grow in a temperature range of 10-45°C and in a pH range of 4.2-9.3 (Asperger, 1994).

Staphylococcus aureus organisms produce an extra-cellular polysaccharide (EPS) capsule, and they also have the ability to involute into the epithelial cells, produce exotoxins and cause tissue necrosis (Weimer, 1998). Some *S. aureus* strains have the ability to resist phagocytosis or to avoid recruitment by polymorphonuclear neutrophils (PMN).

Staphylococcus aureus interacts strongly with the immune system both during the invasion and during the recovery process. Adhesion of the bacteria to epithelial cells is a very important step during the intramammary infection. Recently, research has shown that success of the adhesion is probably related to the bacteria's genetics, since it could

be regulated by a plasmid that also encodes resistance to penicillin (Zecconi and Smith, 2003).

Dry cow therapy is designed to cure existing infections and prevent new infections that could take place during the dry period. However, *Staphylococcus aureus* differs in its resistance towards antibiotics, among its resistance mechanisms are found: Resistance to β-lactamase antibiotics, tetracycline, sulfonamide, trimethoprim, macrolides and aminoglycosides and plasmid resistance (Zecconi and Smith, 2003).

In order to isolate and identify *Staphylococcus aureus*, many agents can be used such as: tellurite, egg yolk, pyruvate, glycine, fibrinogen, rabbit plasma, NaCl, mannitol and lithium chloride among others (Zecconi and Smith, 2003). However, usually growth on blood agar, and an identification test such as the Gram stain plus the tube coagulase test are adequate to differentiate the bacteria.

5.4. Organic Dairy Production

Recently, a public concern about renewable resources, soil, water, animal welfare and air pollution has grown. This has increased the interest of producers for new markets, creating a greater involvement in production systems that do not use synthetic materials, such as organic production. According to the Codex Alimentarius Commission and the Joint FAO/WHO Food Standards Program, organic is defined as "a holistic production management system which promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs and where possible, cultural, biological and mechanical methods, as opposed to using synthetic materials" (USDA, 2000).

Over the last decade, the organic industry has increased by about 20% per year around the world. Currently Australia, the European Union, and United States are the largest organic producers in the world, but interest in organic production is increasing in many other countries. In 2003 the organic market was valued at around 23 billion US dollars, and key markets for organic products are the European community, USA and Japan. (USDA, 2000).

Approximately 24 million hectares around the world are managed in an organic way and Australia is the country with the largest area of organic production (around 10 million hectares), followed by Argentina (around 3 million hectares) and Italy (around 1.2 million hectares). In those areas, the two first countries present extensive grazing lands. Nevertheless, the highest proportion of land under organic management is in Europe (Willer and Yussefi, 2004), where the biggest organic market is for fruits and vegetables with 40% of the share, followed by dairy products with 18% (Smith, 2000).

Denmark and Austria are important producers of organic dairy products, with 20% of the milk produced organically in Denmark. It is expected that in a few years half of the milk consumed there will be organic. However, in Austria, due to an oversupply, just 40% of the organic milk is sold as such and the rest is sold as conventional milk (USDA, 2000).

In France, income from organic dairy products was expected to be $133 million in 2003 and is one of the fastest growing areas of the French organic industry, with an expected growth rate of 23% over the next 4 years (USDA, 2000). In addition to that, the increment in the premium price from organic milk and the fall in the regular milk price have encouraged many farmers to convert to organic methods.

In New Zealand, organic production has been increasing recently, especially due to the good image that it brings to New Zealand's agricultural products, which are recognized for their high quality. Conversion of dairy and livestock farms into organic production methods was slower in the past but is now encouraged by the industry, especially for small herd owners that can increase the value of their product without increasing their herd size.

In addition, New Zealand's food industry is dependant on exports and this forces the industry to adapt to new consumer trends. Nowadays, exports of organic products from New Zealand account for approximately 70 million NZD per year with a growth rate of approximately 10% annually (Mason, 2004).

The rules for organic dairy production in New Zealand include: on-farm presence of storage containers for collection and spreading of solid and liquid livestock effluents; non-use of genetically modified products; use of chemically synthesized allopathic

substances (that produce different effects from those caused by the disease itself) in case of failure of phytotherapeutic or homeopathic treatments, but with a period of withdrawal from sale of the product that is twice that for conventional systems; however, use of chemically synthesized allopathic products in a preventive way is forbidden; hormones can be given individually only as a therapeutic veterinary treatment; animals should be fed with organically produced feeds, however up to 10% of the dry matter fed annually to the animals could come from a non-organic source (Agriquality, 2003).

Animals (and their products) treated with chemically synthesized allopathic remedies more than two and a maximum of three times within the same year will not be sold as organic, and will have to go through a conversion process (Agriquality, 2003).

At the present time, there are about 66 dairy farms certified as organic or in the process of becoming certified around New Zealand (Stevenson, 2002).

5.4.1. Approach to mastitis

In general, organic systems are based on the idea of a more sustainable approach to agricultural procedures. Organic methods try to nurture the ecosystem by controlling pests, weeds and diseases preferably with dependent life forms; to recycle animal and plant residues; to use crop selection, rotation, tilling and cultivation. Also, to enhance soil biological activity, and to manage pests and diseases through encouragement of host-predator relationships (Agriquality, 2003). As an illustration, they try to maintain and increase soil fertility and animal health by renewable resources, and by promoting non-chemical disease prevention instead of reliance on curative treatments (Thambsborg *et al.*, 1999).

Therefore, control of animal health and welfare play an important role in this system. Organic dairy production is based on a preventive approach, with the help of selected breeds or strains, which uses high quality diets and tries to improve the productive environment and reduce all kinds of stress in the animals. Since the use of chemically-synthesized allopathic medicinal products is not allowed, animals do not receive growth hormones or antibiotics; however preventive practices, like vaccines, are accepted (Zwald et al., 2004). When animals are injured or sick, the use of phytotherapeutic and homeopathical products is suggested (Agriquality, 2003).

Phytotherapy is an old principle that relates to the use of herbs or herbal products, based on the idea that plants have been the basis of many medicaments and still are, but not in a refined way, as in chemical-synthesized compounds.

Homeopathy is based on the principle of "like cures like", meaning that the substance that produces the symptoms in a healthy individual will cure the symptoms in a sick individual which presents similar symptoms (Verkade, 1997). Remedies are prepared based on individual cases and "potentialized". This is a process of dilution by 1 to 100 (centesimal dilution scale). In New Zealand, the common potency is 30c, where the toxic substance is diluted 1 in 100 thirty times. Most commonly used homeopathical remedies for mastitis and their indications are shown in Table 5.1.

Table 5.1 Most common Homeopathical mastitis remedies

Remedy:	Indicated for:
Belladona	Acute post calving cases with a swollen, red udder
Aconite	Acute sudden cases
Apis Mellifica	Fresh calved heifers with edema
Bryonia Alba	Hard swollen udder
Arnica Montana	Mastitis developed as a consequence of a trauma on the udder tissue
Bellis Perennis	For deep injuries
Phytolacca	For acute and chronic cases, where milk shows curd and clots
Urtica urens	In cases of Edema
SSC (Sulphur, Silicea and Carbo veg.)	Excellent results in acute and chronical cases, where yellow clots show, especially in the first squirts of milk
Ipecac	Useful in cases of intramammary bleeding

(McLeod, 1981)

In addition to regular homeopathical treatments, since organic procedures focus on a preventive basis, mastitis nosodes are commonly used. A nosode is a product obtained from a part of the diseased individual, commonly from a lymph node or secretions, which are a response to the illness and are used to prepare a vaccine for the particular causative organism. Nosodes are commonly added to the water supply as a preventive measure.

In 1997, a trial with ten Vermont farms tested nosodes to evaluate their efficacy in reducing the incidence of mastitis in the herds. The nosodes were prepared commercially, and were based on milk samples from cows with clinical mastitis on the participating farms. Nosode and placebo were diluted in a 50% alcohol solution and sprayed in the mucosa of the vagina first for five consecutive days and later once every two months to all the animals and after calving and dry-off for the lactating ones (Barlow *et al.*, 2001).

Results were evaluated by culturing samples taken at calving, 30 days after it, at dry-off, before any treatments when clinical cases showed up and 30 days after treatment. There were no differences in the rates of new infections between nosode treated and untreated animals (Barlow *et al.*, 2001).

Likewise, different studies have analyzed the effects of this approach, but still the data coming from scientific research of homeopathical treatments is limited and no definite conclusions can be stated.

5.4.2. Intramammary infections in Organic herds

As previously mentioned, mastitis represents an important ongoing problem in organic herds. Most of the research done in organic production systems has taken place in Scandinavian countries, mainly in Denmark, Switzerland and Sweden, but also in England and Wales. It has been mainly oriented towards description of mastitis patterns, treatments used by farmers and their responses.

In 1996, Weller and Cooper collected data from 11 organic herds in England and Wales for a period of two years. (The farms had converted or were in the process of converting to organic). Clinical mastitis was the main health problem in all the farms. Even though somatic cell counts were generally under the 400,000 cells/ml penalty mark, three farms had an increasing cell count, showing an increase in the incidence of subclinical mastitis.

During the first year, 70% of the clinical cases were treated with alternative remedies, and 65% in the second year. In eight of the farms, antibiotics were used to treat the most severe clinical cases. Finally, it was observed that despite the use of nosodes, the

incidence of mastitis was higher than had been reported previously for other organic farms, and for most conventional farms (Weller and Cooper, 1996).

In 1997, Vaarst and Enevoldsen collected data for clinical signs, inflammatory reactions and microbiological identifications from 367 cases of clinical mastitis during 18 months in 14 organic dairy farms in Denmark in order to describe the pattern of clinical events.

Of the clinical cases recorded, 20% were bacteriologically negative. From cases with bacterial growth, just 6% were caused by coliforms and were usually located in just one quarter; 18% of the cases were positive for *Staphylococcus aureus* and were more frequently (42%) found in late lactation and near dry-off. *Streptococcus dysgalactiae* accounted for 9% of the cases and *S. uberis* for 23% (Vaarst and Enevoldsen, 1997).

In 1998, Hovi and Roderick collected data about prevention and treatment of mastitis in 16 established organic farms in England and Wales (and compared them against 7 conventional farms), in order to advise producers about results of different organic farming procedures. A total of 960 cases of clinical mastitis were recorded in all herds. From those, 61% were treated with antibiotics (41% of the cases in the organic farms were treated with antibiotics) and 52% of all the treatments in the organic farms used homeopathy. In the organic farms 16% of mastitis cases appeared during the dry period (Hovi and Roderick, 1998).

Busato *et al.* (2000), performed California mastitis tests (CMT) and bacteriological follow-ups (for CMT> 1+) in 152 organic farms around Switzerland to estimate the prevalence and etiology of subclinical mastitis cases in that country. The prevalence of subclinical mastitis was 21% between day 7 and 100 of lactation and 35% from 101 until 305 days in milk. Also, a decrease of *S. aureus* and an increment of *C. bovis* were seen between early and late lactation (Busato et al., 2000).

Hardeng and Edge in 2001 investigated differences in disease incidence between conventional and organic herds in Norway, where 31 organic herds were compared against 93 conventional herds. On average, cows in organic farms were older than in conventional herds (2.97 lactations vs. 2.35 respectively). In addition, animals in the organic farms were culled at an average age of 5.3 years in organic herds compared with 4.5 years in conventional farms. Furthermore, SCC was significantly higher in organic

farms than in conventional herds, but lower in the first two lactations and then significantly higher after the sixth lactation. However, the number of cows with a high mean SCC (>200,000) through the lactation divided by the total SCC (total number of counts) was not different between productive systems, concluding that subclinical levels were similar in both groups (Hardeng and Edge, 2001).

In New Zealand, the trial that is the subject of this thesis started in 2001 to monitor differences between organic and conventional dairy farming in two farmlets. Results from the first year of trial before certification, showed a slightly higher somatic cell count for the organic herd, but with no significant differences between herds. In addition, frequencies of clinical mastitis were similar as well, with 16% in the organic and 14% in the conventional herd (Lopez-Villalobos *et al.*, 2003).

In Denmark, from 1997 to 2001, data from 48 organic herds was analyzed to estimate effects of somatic cell counts and mastitis treatments on milk production. Results showed that milk losses due to these causes in organic Danish herds were similar to losses in conventional herds in other countries (Bennedsgaard *et al.*, 2003).

In 2002, data from 26 organic dairy herds in Sweden was analyzed and compared against data from 1102 conventional herds. Results showed that mastitis was the disease that required the most veterinary calls. However, the mean of veterinary treatments per 100 cow-years calculated as (number of cases/number of cow-days for the herd)*365*100 was 9 in organic herds compared to nearly 15 in conventional herds (Hamilton *et al.*, 2002).

However, these results could be a consequence of a tendency from organic farmers to call the veterinary less often, which could lead to a welfare concern, since animals are not treated properly according to their infections to avoid milk discarding or quarantined animals.

5.5. Prevention of Dry period intramammary infections

5.5.1. Conventional procedures

In order to control the problems associated with the dry period (section 5.1), prophylactic methods to protect the mammary gland during the dry period such as dry cow therapy, are widely used in conventional dairy farms. Dry cow therapy has a double purpose: to eliminate existing infections and to prevent new infections during the dry period.

Dry cow therapy consists of infusing a long lasting antibiotic into the gland via the teat canal of quarters immediately after the last milking before drying-off. Different strategies are used: DCT could be given to all quarters regardless of the infection status of the animal; to just the infected quarters; or to all the quarters of an animal showing at least one infected quarter or to all quarters of cows which had a high SCC (Browning *et al.*, 1994).

However, dry cow therapy has shown to be ineffective against new infections caused by coliforms in the periparturient phase, when the glands are extremely susceptible (Oliver and Sordillo, 1988).

The incidence of IMI by environmental pathogens during the dry period was studied by Smith *et al.* (1985). 139 cows were sampled two weeks and one week before dry off, after drying-off, prior to calving, at calving and post calving. Dry cow therapy was compared to immunization against coliform bacteria, and a combination of both. Results showed that streptococcal infections remained for a longer period than coliform infections and that alternative methods to conventional dry cow therapy should be used for controlling coliform infections (Smith *et al.*, 1985).

In New Zealand, dry cow therapy is commonly used to treat existing infections at drying-off. In 1986, a survey done with 2460 dairy herds showed that in 81% of them it was used in a selective way, based on records of high somatic cell counts and clinical mastitis cases through the season (Laycock *et al.*, 1987).

Further research questioned the prophylactic effect of dry cow therapy on *Streptococcus uberis*. Animals from four farms were sampled one week before drying-off and from one to four days after calving. During the dry period, quarters were palpated and if

clinical mastitis was considered to be present, samples were taken. Treatments used were: Non-infected cows given no treatment (NI-NT) compared to non- infected cows with all quarters treated (NI-AT) and infected cows with infected quarters treated (I-IQ) compared to infected cows with all quarters treated (I-TC).

In general, results showed an incidence of new intramammary infections (IMI) during the dry period of 4% and of 7% after calving for all groups combined. Bacteria isolated from the dry period samples were as shown in Table 5.2 and the only significant differences ($P<0.01$) between the groups were for *S. uberis* infections, which accounted for must of the infections, therefore total new infections were significantly different as well.

Table 5.2 New IMI's at dry-off from the Williamson et al. trial (1995) and the percentage of those infections caused by each type of bacteria

Bacteria Isolated	NI-NT	NI-AT	I-IQ	I-TC
New IMI's at dry-off	7	1	7	0
Streptococcus uberis	91	72	100	0
Staphylococcus aureus	6	28	0	0
Minor pathogens (CNS and *C. bovis*)	3	0	0	0

Dry cow therapy was related to a lower incidence of *Streptococcus uberis*, with 12.3% of infections in untreated quarters and 1.2% in DCT quarters. However, infections due to *Staphylococcus aureus*, Coagulase-negative staphylococcus and *Corynebacterium bovis* were not significantly different between the animals treated with dry cow therapy and the untreated ones. Cure rates for infections existing at dry-off were similar in both I-TQ and I-TC groups and are shown in Table 5.3 (Williamson *et al.*, 1995).

Table 5.3 Cure rate for existing infections obtained from Dry Cow Therapy (DCT) by Williamson et al. (1995)

Bacteria	Cure (%)
Staphylococcus aureus	79
Streptococcus uberis	78
Minor pathogens (CNS and *C. bovis*)	88

In addition, 83% of all infections occurring during the dry period took place during the first 21 days after dry-off. Moreover, 97% of the quarters with a new infection were quarters that had an open teat at that stage. It was observed, that during the first fifty days, untreated animals showed a higher rate of open teats compared to treated animals. However, after this period all treatments showed a similar rate of open teats (Williamson *et al.*, 1995).

At calving similar results were present, where the percentage of new intramammary infections were significantly different between groups ($P<0.01$). *Streptococcus uberis* infections were significantly different between the groups with no infections at dry-off, with a higher percentage of infections in the non-treated group. However, results were similar for the groups with infected animals at dry-off.

Nevertheless, due to public concern related to the excessive use of antibiotics, effectiveness of selective dry cow therapy has been tested in different trials. In 2001, four herds, two conventional with low SCC (236 animals in total) and two converting to organic (79 animals in total) participated in a trial where half of the animals were treated with DCT and half did not receive any treatment. Animals were sampled one week before drying-off, at drying-off, within 24 hours of calving, one and two weeks after parturition. Results showed that the most common pathogen at calving was *S. uberis* for both systems. However, for conventional herds the second most common were coliforms. There were significantly more infections at calving for untreated animals in both production systems than for treated animals, and the organic herds showed a higher prevalence of *S. aureus*. Incidence of infections during the dry period in untreated quarters was 1.5% in the conventional herds and 4% in the organic groups (Berry and Hillerton, 2002b).

In New Zealand, the SAMM plan recommends that farmers should review cell counts and clinical records before drying animals off to decide whether to cull or treat them with DCT. The herd should be classified as one with a high or low incidence of mastitis, the first being a herd with 40% or more of the cows with a SCC level higher than 120,000 (for heifers) or of 150,000 (for cows), or if 10% or more of the herd had clinical mastitis in the previous dry period or in the first month of lactation. In "high" mastitis incidence herds all cows should be treated with DCT whereas "low" incidence herds only selected animals should be treated. In addition, to avoid problems in the

establishment of the teat keratin plug, an abrupt drying-off is suggested, where feed intake is restricted and animals are milked once a day for two to three days to reduce production before the final abrupt dry-off (Joe, 1993).

Likewise, milking equipment should be tested and repaired during the dry period and heifers should be trained to go into the milking shed. Afterwards, the cows should be calved in clean pastures and teats should be checked for clinical signs during the colostrum period. After calving, teats should be sprayed with an iodine teatspray containing an emollient for the entire lactation to reduce contamination and to minimize teat soreness and damage. Finally, clinical cases should be identified quickly and separated from the main herd by being milked last to avoid spread of infections by these known infected cows (Joe, 1993).

5.5.2. Organic procedures

Organic systems are strongly based on prevention of diseases. Therefore, hygienic practices during milking, like the ones stated in the SAMM plan, become especially important. In addition, as previously stated, the natural keratin plug formed inside the teat canal is an important factor that protects the teat from new infections during the dry period, since it has been shown that "closed" teats rarely present new infections during the dry period. Therefore, in order to protect those quarters which do not have a plug, external teat sealers were created and tested in different trials. However, those external sealers did not remain attached to the teat for long enough, so that internal teat sealers emerged as the product which could provide a long-lasting physical barrier in the teat-cistern while the natural keratin plug was being formed.

Early research in Ireland during the late 1970s used an antibiotic-free inorganic salt in a paraffin-wax base. Animals free of infection were assigned either to a teat sealer treatment or to treatment with antibiotics and teat sealer, treating just two quarters in each udder with the assigned treatment and leaving 2 quarters as a negative control. Infected animals were treated with antibiotics and teat sealer except in one quarter which was left as a control (Meaney, 1977).

Animals were sampled one week before dry-off, 3 days later, at dry-off, at calving and one week post calving. In addition, teats were "challenged" during the dry period by being dipped in a bacterial culture every week.

For the infection-free animals, results showed an IMI incidence of 3.5% and 5.8% for teat sealer and teat sealer with antibiotic respectively against 32% for the negative control. For the infected animals, data showed a reduction in new infections of around 57%, a cure rate similar to the regular dry cow therapy procedure. Finally, a persistence of the seal in the teat ducts of around 3-4 weeks was observed (Meaney, 1977).

Later, a new formula of teat sealer was developed and used in New Zealand. In 1998, three herds with an incidence above average of clinical mastitis during the dry period were studied. Just quarters without infections were included in the study. On each cow one quarter was left untreated as a negative control and another was infused with dry cow antibiotic as a positive control. The third and fourth quarters were treated with teat sealer (Teatseal, based on bismuth nitrate) and antibiotic followed by a tube of teat sealer (Woolford *et al.*, 1998).

Milk samples were taken within seven days before dry-off from all quarters; from every clinically affected quarter recognized by palpation during the dry period; and from all quarters within 24 hours after calving.

Results for the treatments and positive control showed a reduction of new intramammary infections of around ten times compared to the negative control. However, there were no significant differences among treatments. In addition, *Streptococcus uberis* was the bacteria most isolated at calving (Woolford *et al.*, 1998).

Berry and Hillerton used the same teat sealer in United Kingdom conditions with two conventional herds, two fully organic herds and three herds going into organic production. Animals were sampled one week before dry-off, at dry-off, at calving and one week post-calving. Results showed that animals treated with teat sealer presented less new infections by 0.27 times, than the non-treated group (Berry and Hillerton, 2002a).

In New Zealand, teat sealers are allowed to be used in organic production systems as a preventive procedure during the dry period for new mastitis infections. However, no comparison has been done between established organic and conventional herds during the dry period, where conventional animals are subject to an antibiotic treatment and

organic animals to a teat sealer in order to prevent further infections in the following early lactation.

5.6. Mastitis detection and diagnosis

Since mastitis is an inflammation of the mammary gland, detection and diagnosis can be performed by regular monitoring of the udder condition, however, this procedure can only detect clinical mastitis cases. Therefore, to detect subclinical cases, indirect methods must be used. Among those are included: high somatic cell counts (SCC), increment in milk conductivity, increment in milk enzymes and decrement in percentages of milk components like fat, lactose and casein (Hogan *et al.*, 1999).

Diagnosis on the other hand, requires identifying the causative agent of the infection. Microbiological examinations are then performed in milk samples to isolate the responsible bacteria. This can be done at a herd level using bulk tank samples or at a cow level, sampling individual quarters or composite milk samples from all quarters (Hogan *et al.*, 1999).

Samples can be taken as a single sample, duplicated (two samples taken at the same occasion, which involves sampling once, cleaning the teats again with alcohol and then sampling a second time) or on consecutive days (sampling with at least one day of interval between them).

In most commercial dairy farms multiple cultures are not practical, and diagnosis must then rely on a single culture since it is practical when handling many samples. If additional samples can be taken of questionable samples or highly suspected animals, then uncertainties can be reduced (Sears and McCarthy, 2003a). The National Mastitis Council sampling protocol mainly highlights the importance of an aseptic sampling process, where contamination is reduced and therefore results are reliable. However, just when testing preparations for treating mastitis, the American Food and Drug Administration (FDA) guidelines require duplicate or consecutive samples.

As a consequence of the use of different number of samples, researchers have tested the use of one or more samples for mastitis diagnosis. Jasper *et al.* showed that an

agreement of 96.2% was obtained between duplicate samples, giving the single sample an error of less than 5% (Jasper *et al.*, 1974). Nevertheless, the importance of a good sampling technique was highlighted and it was stated that a higher accuracy would be obtained with duplicate or consecutive sampling.

Further observations with pairs of quarter milk samples showed a disagreement between duplicate samples of 4.5% and of 11.5% from consecutive samples with two to seven days of interval. *S. aureus* infections showed a disagreement of 1.6% for duplicate samples, 2.7% for consecutive samples with 2-7 days interval and 6.9% for 8-16 days interval. Disagreement for *S. agalactiae* was of 1.1%, 6.6% and 6.9% respectively. It was concluded that a single sample could have an error as low as 3% (Postle, 1976).

Supporting data showed an agreement between duplicate samples of 96.4% and of 94.2% for *Streptococcus agalactiae* and *Staphylococcus aureus* respectively. It was estimated that single quarter samples would have identified around 98% of *S. agalactiae* and around 97% of *S. aureus*. For other pathogens the agreement was lower, with 55.6% for coliforms and streptococci other than *S. agalactiae* 81.6%. Finally, it was stated that single samples could be accepted as an identification procedure for *S. agalactiae* and *S. aureus* (Erskine and Eberhart, 1988).

However, more recent research has shown that the use of single samples for *S. aureus* equals the mean sensitivity of the infected glands, which depends on the degree of bacteria shedding of each one. Two shedding cycles were found for a specific *S. aureus* isolate used for a bacterial challenge, and from 991 consecutive samples taken, 745 were positive, obtaining a sensitivity (probability of a true positive) range for each gland from 41 to 100%. The sensitivity of a single sampling was of 70% ± 13.5% for a low shedding cycle and of 100% for the high cycle glands (Sears *et al.*, 1990).

Overall sensitivities were presented for a single quarter sample of 74.5% ± 16.75% and of 94 and 98% for two and three consecutive samplings respectively. However, in the case of natural intramammary infections, it was found that single quarter samples sensitivity ranged from 63 to 100%, that is, the sensitivity for detecting *S. aureus* with a single sample at any point would be of 89% ± 17.5%. It was concluded that without knowledge of the shedding cycle of the isolate, accuracy of both single and duplicate samples could give doubtful diagnosis (Sears *et al.*, 1990).

Later, Dinsmore *et al.*, found that in the case of *Streptococcus agalactiae* the sensitivity for single cultures was in the range of 95- 100% and that around 10% of cultures (either positive or negative) were usually misclassified. However, in the case of a pathogen eradication plan, repeated herd sampling was suggested (Dinsmore *et al.*, 1991).

Finally, when possible, duplicate samples should be suggested to obtain a more accurate result, however, single samples could give a proper diagnosis, with additional samples in doubtful cases.

5.7. Conclusions

The importance of mastitis control and prevention has been highlighted over the years in the dairy industry. In organic systems it becomes a sensitive area, since it is considered the most important health related problem in those productive methods, and because antibiotics as important curative and preventive tools, cannot be used easily.

Previous research in the area has described infection patterns in organic herds and the different treatments and preventive procedures, but mainly in Scandinavian countries and the United Kingdom. Data in New Zealand is limited, despite the increasing importance of these methods in New Zealand.

However, research in New Zealand grazing systems shows a distinctive mastitis infection pattern, where most of the infections appear around calving due to environmental pathogens, mainly, *Streptococcus uberis*. Later in lactation, it has been found that Coagulase negative staphylococcus and *Staphylococcus aureus*, a contagious pathogen, increase in importance.

In New Zealand, Dry Cow Therapy (DCT) is the most effective method available for controlling and preventing new infections during the dry period. It is usually applied on a selective basis, where animals with a high SCC and clinical mastitis records are treated. In addition, research has proven that teat sealers, inorganic salts that create an internal barrier inside the teat, are as effective at preventing new intramammary infections during the dry period as Dry cow therapy and are currently authorized for their use on organic farms.

Data from an organic trial at Massey University, New Zealand, showed no significant differences in somatic cell counts and a similar incidence of clinical mastitis cases between a conventional and an organic farm after one year of beginning the conversion process to become an organic system.

Nevertheless, more research in New Zealand is required to provide more information on pathogens important for the increasing number of organic producers in order to improve the current preventive and therapy practice of those farms.

6. MATERIALS AND METHODS

6.1. Study design

The study was planned in March 2004 to monitor the mastitis status of two experimental herds (organic and conventional) at the Dairy Cattle Research Unit (DCRU) in Massey University and to detect any differences between them. Samples were taken on four occasions: Mid-lactation (Nov 03), before dry-off (Apr-May 04), at calving and 14 days after calving (Aug-Nov 04), in order to detect the prevalence and type of infections in all quarters of all cows.

6.1.1. The farmlets

In August 2001 a trial started at the Dairy Cattle Research Unit in Massey University (Palmerston North, New Zealand), where half of the 40 ha farm started to convert to organic certification, while the other half continued to be managed conventionally. The existing herd at that point was divided as equally as possible into two separated herds, based on breeding worth, production worth, weight, age and somatic cell count. The objectives of the trial were to quantify differences in production of milk solids per cow and per hectare; to compare EFS shown by the two systems during the first five years; to identify significant differences in animal health issues; to document key issues during the conversion process and the best management options for the organic herd.

Both herds share the same milking shed and staff but with a strict management control, where the organic herd is always milked first and any animals treated with antibiotics are quarantined and left to be milked last after all the other cows. Two separate milk tanks are used. Conventional chemicals used to clean the pipes and milking machines are allowed by the organic regulations in order to avoid any contamination of the milk. Teats of cows in both herds are sprayed immediately after each milking with a sanitizer containing sodium iodide (Teat guard plus, Ecolab, Hamilton NZ). During the lactation that began in August 2004, the teat-cups were rinsed with acid sanitizer after the organic herd was milked.

Results from the first year of the trial showed that the average somatic cell count (SCC) was slightly higher for the organic herd than for the conventional herd, but the difference was not significant. The incidence of clinical mastitis cases was similar in both herds, with a frequency of 15.9% for the organic and 13.6% for the conventional (Lopez-Villalobos *et al.*, 2003).

In 2003, when the present study started, the organic farmlet became fully certified and obtained the 10% premium price given by Fonterra to organic milk producers.

At the end of season 03-04 animals received treatments according to different conditions in both herds. In the organic herd, animals with previously known mastitis problems and/or high SCC were not treated at all since antibiotics are forbidden in organic systems. However, animals with a SCC lower than 150,000 cells/ml in the last herd test and with no records of mastitis during the lactation, were entitled to be treated with teat sealer (Teatseal, Pfizer, Auckland NZ) after the final milking to prevent infections in the dry period. In the conventional herd, animals with a SCC above 150,000 cells/ml or with clinical mastitis received Dry Cow Therapy (DCT) to cure known infections and the rest of the animals were not treated since no infections were present. Consequently, treatment groups were biased, either DCT (Dryclox DC, Bomac Laboratories Ltd, NZ) for high SCC cows in the conventional herd, or teat sealer for low SCC cows in the organic herds. However, changes in infection status are described and analyzed after calving following the most common practice in each system.

6.2. Milk Sampling

Foremilk samples were collected on four occasions between November 2003 and November 2004: During mid lactation (November 2003); at the last milking before dry-off (April-May 2004); at the first milking or within 24 hours of calving (July-October 2004) and again 14 days post calving (August-November 2004).

6.2.1. Number of samples

The use of single quarter samples was the most feasible option since there was limited staff at the moment of dry-off. In addition, laboratory procedures were carried just by

the author and single samples were more practical to handle due to the number of cows involved. To increase accuracy, in the case of doubtful results, animals were sampled again a day after the first sample, except for the samples at dry-off.

Frozen samples from a previous trial conducted in November 2003 with the same animals and the same staff provided the mid-lactation samples. The sampling protocol was obtained from the National Mastitis Council and followed the same procedures in all samplings. Number of animals was limited by the size of both herds, and therefore all animals were sampled.

A total of three people sampled animals during the four sample periods. However, all followed the same protocols for sampling.

6.2.2. Sampling Protocol

According to the protocols of the National Mastitis Council (Hogan *et al.*, 1999). On each occasion, a few milliliters of foremilk were collected using aseptic methods, as follows: Sterile plastic bottles with a plastic screw cap and identified with a waterproof marker were used to collect the samples, and the technician wore rubber gloves during sampling.

Cotton swabs soaked in a 70% isopropyl solution (methylated spirits) were used to clean each teat. Each quarter was cleaned carefully, trying to leave the opening of the teat canal with no signs of contamination and as clean as possible. Front quarters were cleaned first and rear quarters second.

Teats were allowed to dry before the samples were taken. Back quarters were sampled first and front quarters second. First, a few squirts of milk were discarded in order to clean the teat canal. Afterwards, the bottles were opened while keeping the cap in the same hand close to the body of the bottle in order to avoid contamination, and were kept as horizontal as possible while taking the sample. Samplers avoided touching any areas with the bottle while sampling and bottles were closed immediately after sampling.

Samples were then kept under refrigeration until they were plated at the laboratory, which was at most four hours after they had been taken, with the exception of mid lactation samples, which were kept frozen at -20° C from November 2003 until June–

September 2004, before being cultured. The latter were thawed at 37°C and then cultured with the same methods used for the rest of the samples.

Effects of short periods of freezing on milk pathogens have been analyzed, and the results have shown no changes up to 6 weeks and a decrease in *E. coli* and *Actinomyces* after 14 weeks with an increment in coagulase-negative staphylococci. Researchers concluded that freezing samples before analyzing them did not seriously compromise the results at the laboratory (Murdough *et al.*, 1996). However, periods of freezing as long as the one in the present study have not been analyzed before.

6.3. Bacteriological analysis

Milk cultures were done following the National Mastitis Council (NMC) procedures (Hogan et al., 1999): 0.01 ml of milk was deposited on an aesculin blood agar plate, with 1gr of aesculin per 1000 ml of blood agar (Fort Richards, Auckland NZ) with a pipette and then streaked with a loop. Plates were kept in an incubator at 36-37 °C for at least 18 hours before the first reading and were re-checked at most 48 hours after plated.

The growth and physical characteristics of colonies were recorded for every plate. Since contaminants usually grow faster and larger than pathogenic staphylococci and streptococci, a high importance was given to hygiene at sampling. For all bacteria, the presence of more than three similar colonies was considered a positive infection. However, any growth of *Streptococcus agalactiae* and *Staphylococcus aureus* was always taken to show an intramammary infection (Sears and McCarthy, 2003a). All bacteriological analyses were performed by the same person; therefore, the same criterion was given to each quarter sample analyzed through all sample periods.

Staphylococci are common pathogenic species isolated from milk and as mentioned before, *Staphylococcus aureus* is the main cause of mastitis in many herds. To identify them, a catalase test was conducted on each different colony found on the plates, where a colony was taken carefully from the agar with a loop, trying to avoid touching the blood agar and then spread on a glass slide. One drop of peroxide at 3% was poured on top of the colony and if a reaction creating bubbles was obtained, the test was

considered positive, and the colony was classified as Staphylococci. To confirm this, a smear was done and later Gram stained, checked under the microscope and recorded. Purple cocci were expected for both species, but arranged in clusters for Staphylococci and in chains for Streptococci.

6.4. Bacterial identification procedures

The most practical way of classifying *Staphylococcus* is: a) *Staphylococcus aureus*, which is a distinctive colony with a complete or incomplete hemolysis showing a pigment and testing positive in the tube coagulase test and b) Coagulase-negative (CNS or non coagulase) staphylococcus, which show very little or no hemolysis and are negative for the tube coagulase test (Hogan *et al*., 1999).

For detecting *S. aureus* strains, the tube coagulase test was used, according to NMC, which consists of depositing one suspected colony in a glass tube containing 0.5 ml of rabbit plasma coagulase (Ngaio Laboratories) and then incubating it at 35-37°C overnight. Coagulated tubes were considered positive and the colony classified as *Staphylococcus aureus*, negatives were recorded as Coagulase-negative staphylococci (CNS).

In the case of catalase negative colonies, if the aesculin was positive (black agar under the colonies) an inulin and Bagg broth test were performed. First, a purity plate was grown, where the suspected colony was plated on sheep blood agar (Fort Richards, Auckland NZ). 24 hours later, a colony was deposited in a glass bottle containing an inulin solution (Fort Richards, Auckland NZ) and another in a bottle containing Bagg broth (Fort Richards, Auckland NZ). Bottles were incubated at 35-37° C overnight and checked for a color change reaction. In cases of no reaction or partial reaction, bottles were re-checked at 24 and 48 hours and then results recorded.

If the inulin changed from pink to yellow, the test was considered positive. However a confirmatory test was used and if the Bagg broth showed no changes in color the colony was identified as *Streptococcus uberis*. In the case of a partial or negative reaction in the inulin (orange-pink) and a color change in the Bagg broth from purple to brown, the colony was identified as *Streptococcus* spp.

In the case of catalase negative colonies with no reduction of aesculin, a purity plate was grown and 24 hours later a CAMP test was performed, where a Columbia sheep blood agar plate (Fort Richards, Auckland NZ) was warmed at an incubator at 37°C and then plated with one horizontal streak of *S. aureus* and a perpendicular streak of the colony analyzed without touching the previous streak. Two control colonies were plated parallel to the suspicious colony: *S. agalactiae* and *S. dysgalactiae*. The plate was then incubated at 36-37° C overnight. An arrow shaped hemolysis takes place in the case of a positive result, which will be the same as that obtained by the *S. agalactiae* (Murphy *et al.*, 1952).

In case of a negative result in the CAMP test, the colony was classified just as *Streptococcus* spp.

For all the samples taken at calving and 14 days post calving, in cases which showed possible contamination with a Gram negative organism, a second sample was obtained the day after the first sampling and if that same pathogen was isolated, the animal was considered to be infected with it.

When Gram negative organisms were found, colonies were subcultured on McConkey agar to check their reaction to lactose. Just two Gram negative organisms were isolated: *Proteus* and *Escherichia coli*. Swarming effect in plates was used to identify *Proteus* species. *E. coli* was identified by oxidase, citrate and indole tests. However, an identification Api 24 strip (Biomeriux, France) was used to confirm results.

6.5. Somatic cell counts and milk solid yields

Milk from animals with high SCC, especially in the organic herd, was not collected in the tank with the rest of the milk from the herd to avoid a penalty SCC level. Therefore, data from the bulk milk tank was not used for statistical analysis. Monthly individual herd tests were then used for both herds. Data was recorded individually each month and analyzed for both seasons separately. Milk solid yields from the herd test dates were also recorded and analyzed.

6.6. Statistical analysis

Due to animals being culled at the end of season 03-04 and the entrance of replacement heifers to the herds at the beginning of season 04-05, the number and identity of animals was different at each sampling. Therefore, data was analyzed separately for each sampling period using the PROC GENMOD procedure of SAS 8.02 (2001) with a logit function. The model considered the effect of herd and age of the animals, which was divided into three groups: 2-3 years old, 4-5 years old and above 5 years old. At the quarter level the effect of quarter location was considered as well. Models were run for each different bacteria. For cows, the model considered the herd, age of the animal and interaction of herd and age. For quarters, the model considered the herd, quarter location, age of animal and interaction of herd and age.

Individual somatic cell count data from monthly herd tests were subjected to a natural logarithmic transformation (LSCC) and followed a repeated measurement analysis using the PROC MIXED procedure of SAS (2001). The model analyzed the LSCC considering effects that included herd, cow within herd, herd test and the interaction between the herd and the herd test.

Clinical cases were analyzed with 2 x 2 contingency tables using the PROC FREQ procedure of SAS (2001), through a chi square analysis. Frequency of mastitis for each season was defined as the number of cows diagnosed with clinical mastitis one or more times during lactation, divided by the total number of cows in each herd.

Milk solid yields were analyzed with the PROC GLM procedure following a natural logarithmic transformation (LMS) and using SCC as a covariate. The model analyzed the LMS considering LSCC, herd and the interaction between LSCC and herd.

The results of samples from cows which were, or were not treated with either Dry cow therapy or with teat sealer after dry-off, were subjected to simple, descriptive analyses. This was done because the treatment groups were created to be unequal in infection status, according to best common practice for each production system. Treatments were analyzed for the frequency of infection shown in each herd, using contingency tables with the PROC FREQ procedure of SAS and a chi square analysis. In those cases where this analysis was not valid, a Fisher test was performed.

7. RESULTS

Data was analyzed on the basis of quarters and cows, and results are reported for both. The numbers given as quarters with bacterial growth or infected quarters are those quarters in which the milk cultures showed growth (from light to heavy) from any of the bacteria stated. Percentages shown for each specific pathogen are based on the number of samples (quarters or cows) in which the bacteria grew, divided by the total number sampled (quarters or cows).

The number of cows and quarters sampled on each of the four occasions varied as a consequence of some animals being culled at the end of the 03-04 season, and of replacement heifers being introduced to the herds at the beginning of season 04-05. Therefore, the number of animals or quarters for each sample period is stated next to the results obtained. In the organic herd, there were three animals each with one blind quarter; while in the conventional herd one animal presented a blind quarter at dry-off.

The effect of age was analyzed in three groups: 2-3 years old; 4-5 years old and above 5 years old. Animals above five years old showed a higher significant difference in the percentage of infections in mid lactation and also showed a higher percentage of infections caused by *Staphylococcus aureus* in all sampling periods except dry-off. However, due to the low number of animals in each group in each herd, no significant differences were shown between herds, and the data is shown in the Appendix 1.

The effect of position of quarter in the udder was analyzed for the quarter level models, but remained consistent in almost all samplings and for all bacteria through the sampling periods. However, when it showed effects, these were stated next to the data for bacterial growth in quarters.

7.1. Mid-lactation samples

Table 7.1 Total number of cows, ages and average age for both herds in mid-lactation samples

	Number of animals of each age										
Herd	**2 yr**	**3 yr**	**4 yr**	**5 yr**	**6 yr**	**7 yr**	**8 yr**	**9 yr**	**10yr**	**Total**	**Average age**
organic	12	9	7	4	4	4	1	4	0	45	4.3
conventional	9	10	7	4	3	4	7	2	2	48	4.9

The number of cows per herd and per age group sampled in mid-lactation is shown in Table 7.1. At this period, a high proportion of quarters showed no bacterial growth, which could possibly be related to the fact that these samples had been frozen for six months before bacteriology. However, the number of quarters with no bacterial growth was similar in both herds. Of the pathogens isolated, Coagulase-negative staphylococcus (CNS) showed the highest prevalence, but the only significant difference between herds was shown in growth of *Staphylococcus aureus*, for which the organic herd showed a higher prevalence in both quarters and cows. The lowest growth of bacteria for both herds was for Streptococci. The number of quarters showing positive or no growth for each type of bacteria is shown in Table 7.2. Same data for number of cows is shown in Table 7.4.

Table 7.2 Total numbers of quarters sampled, with positive or no growth; LSmeans of these numbers (expressed as % of total sampled quarters), and the significance of the differences between the two herds in mid-lactation samples

	Number of quarters		**Lsmeans (% of total sampled quarters)**		
	Organic	Conventional	Organic	Conventional	significance
Total quarters	177	191			
No growth	116	139	64.2	73.8	NS
Growth of pathogens	61	52	35.8	26.2	NS
Bacterial growth					
Staph. aureus	19	6	7.9	1.7	***
Strep. uberis	3	4	1.7	2.1	NS
CNS	41	41	24.2	21.9	NS
Strep. spp.	2	1	1.2	0.5	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

The effect of quarter location in bacterial growth was analyzed and both herds showed a similar distribution of growth among the four quarters as seen in Table 7.3. Fewer quarters remained free of bacterial growth in the back of the udder, with no interaction between herd and position of quarter.

Tabla 7.3 Lsmeans for no bacterial growth according to the quarter location (% of total quarters) in mid-lactation samples

	Front right	Front left	Back right	Back left	Significance
No growth	75.5^{a}	75.1^{a}	66.6^{ab}	57.8^{b}	*

Lsmeans with different superscript significantly differ; *= p <0.05

Table 7.4 Total numbers of cows sampled, with no infected quarters or infected in at least one quarter; Lsmeans of these numbers (expressed as % of total cows) and the significance of the differences between the two herds in mid-lactation samples

	Number of cows		Lsmeans (% of total cows)		
	Organic	Conventional	Organic	Conventional	Significance
Total cows	45	48			
No growth	13	20	23.0	39.8	NS
Growth of pathogens	32	28	77.0	60.2	NS
Bacterial growth					
Staph. aureus	10	4	18.4	4.5	*
Strep. uberis	2	4	4.2	7.9	NS
CNS	25	25	59.1	53.6	NS
Strep. spp.	2	1	4.6	2.1	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

7.2. Dry-off samples

Table 7.5 Total number of cows, ages and average age for both herds in dry-off samples

	Number of animals of each age										
Herd	**2 yr**	**3 yr**	**4 yr**	**5 yr**	**6 yr**	**7 yr**	**8 yr**	**9 yr**	**10yr**	**Total**	**Average age**
organic	12	8	7	4	4	4	1	4	0	44	4.4
conventional	9	11	7	4	3	5	8	1	2	50	4.9

The number of cows per herd and per age group sampled before dry-off is shown in Table 7.5. The proportion of quarters showing no bacterial growth was the lowest out of

all sampling periods, and CNS had the highest prevalence in both herds. The only significant difference was for growth of *Staphylococcus aureus* in quarters, with the organic herd showing higher levels than the conventional herd. However, no significant differences were shown for cows (Table 7.7). The lowest bacterial growth was for *Streptococcus agalactiae* and *Streptococcus uberis.* The number of quarters showing positive or no growth for each type of bacteria is shown in Table 7.6.

Table 7.6 Total numbers of quarters sampled, with positive or no growth; LSmeans of these numbers (expressed as % of total sampled quarters), and the significance of the differences between the two herds in dry-off samples

	Number of quarters		Lsmeans (% of total quarters)		
	Organic	Conventional	Organic	Conventional	Significance
Total quarters	173	198			
No growth	66	68	34.1	33.0	NS
Growth of pathogens	107	130	65.9	67.0	NS
Bacterial growth					
Staph. aureus	19	9	9.9	3.1	*
Strep. uberis	5	2	2.6	0.8	NS
CNS	94	122	54.6	61.6	NS
Strep. spp.	13	7	7.7	3.1	NS
Strep. agalactiae	1	2	0.57	1.01	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

Table 7.7 Total numbers of cows sampled, with no infected quarters or infected in at least one quarter; LSmeans of these numbers (expressed as % of total cows), and the significance of the differences between the two herds in dry–off samples

	Number of cows		Lsmeans (% of total cows)		
	Organic	Conventional	Organic	Conventional	Significance
Total cows	44	50			
No growth	3	3	6.8	6.0	NS
Growth of pathogens	41	47	93.2	94.0	NS
Bacterial growth					
Staph. aureus	11	7	24.7	11.2	NS
Strep. uberis	5	2	10.7	3.2	NS
CNS	40	46	92.5	93.2	NS
Strep. spp.	11	6	25.0	12.0	NS
Strep. agalactiae	1	2	2.3	4.0	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

No effect of quarter location in bacterial growth was observed at this sampling period.

7.3. Calving samples

Table 7.8 Total number of cows, ages and average age for both herds in calving samples

Herd	Number of animals of each age										
	2 yr	3 yr	4 yr	5 yr	6 yr	7 yr	8 yr	9 yr	10 yr	Total	Average age
organic	11	10	7	6	3	3	2	0	3	45	4.3
conventional	11	8	6	7	4	3	3	6	1	49	4.9

The number of cows per herd and per age group sampled after calving is shown in Table 7.8. The highest percentage of growth of *Streptococcus uberis* out of all the sampling periods was presented in both herds. Nevertheless, CNS infections showed the highest prevalence in both herds at this sampling time. However, the only significant difference between herds was observed for *Staphylococcus aureus*, where the conventional herd showed no growth. Gram negative pathogens were isolated from three animals: *Proteus* in one animal that lay down in a stream after a difficult calving and showed a case of septicemia, and *E. coli* in one animal with a very bad udder conformation; both animals were in the organic herd and both were treated with antibiotics. In the conventional herd one animal was positive in one quarter with *Proteus*, it showed general bad health and was treated with antibiotics.

Table 7.9 Total numbers of quarters sampled, with positive or no growth; LSmeans of these numbers (expressed as % of total sampled quarters), and the significance of the differences between the two herds in calving samples

	Number of quarters		Lsmeans (% of total quarters)		
	Organic	Conventional	Organic	Conventional	Significance
Total quarters	177	196			
No growth	100	109	53.6	53.9	NS
Growth of pathogens	77	87	46.4	46.1	NS
Bacterial growth					
Staph. aureus	9	0	4.4	0.0	***
Strep. uberis	15	11	7.2	7.1	NS
CNS	57	74	32.2	37.5	NS
Strep. spp.	8	5	4.6	2.5	NS
Strep. agalactiae	0	1	0.0	0.5	NS
Proteus	3	1	1.7	0.5	NS
E. coli	1	0	0.6	0.0	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

The number of quarters showing positive or no growth for each type of bacteria is shown in Table 7.9. Same data for number of cows is shown in Table 7.10.

No effect of quarter location in bacterial growth was observed at this sampling period.

Table 7.10 Total numbers of cows sampled, with no infected quarters or infected in at least one quarter; LSmeans of these numbers (expressed as % of total cows), and the significance of the differences between the two herds in calving samples

	Number of cows		Lsmeans (% of total cows)		
	Organic	Conventional	Organic	Conventional	Significance
Total cows	45	49			
No growth	11	8	21.2	14.8	NS
Growth of pathogens	34	41	78.8	85.2	NS
Bacterial growth					
Staph. aureus	6	0	13.3	0.0	**
Strep. uberis	11	9	24.9	15.6	NS
CNS	33	39	75.9	80.5	NS
Strep. spp.	6	5	13.7	10.1	NS
Strep. agalactiae	0	1	0.0	2.0	NS
Proteus	2	1	4.4	2.0	NS
E. coli	1	0	2.2	0.0	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

7.4. Fourteen days post-calving samples

Table 7.11 Total number of cows, ages and average age for both herds in samples fourteen days post-calving

	Number of animals of each age										
Herd	**2 yr**	**3 yr**	**4 yr**	**5 yr**	**6 yr**	**7 yr**	**8 yr**	**9 yr**	**10 yr**	**Total**	**Average age**
organic	11	10	6	6	3	3	2	0	3	44	4.3
conventional	11	8	6	7	4	3	3	6	1	49	4.9

The number of cows per herd and per age group sampled fourteen days post-calving is shown in Table 7.11. Positive growth for *Staphylococcus aureus* was shown in the conventional herd. However, the significant difference between herds remained, with the organic herd showing a higher growth of *S. aureus* in both cows and quarters than the conventional herd. Nevertheless, the highest prevalence was still for CNS in both

herds. In this period, a significant difference in growth of *Streptococcus* spp. for cows was found between herds, with the organic herd showing a higher prevalence than the conventional herd. Gram negative pathogens were still present in the same animals mentioned in the organic herd at calving even after the antibiotic treatment.

Table 7.12 Total numbers of quarters sampled, with positive or no growth; LSmeans of these numbers (expressed as % of total sampled quarters), and the significance of the differences between the two herds in samples fourteen days post-calving

	Number of quarters		Lsmeans (% of total quarters)		
	Organic	Conventional	Organic	Conventional	Significance
Total quarters	177	196			
No growth	109	136	63.9	72.0	NS
Growth of pathogens	68	60	36.1	28.0	NS
Bacterial growth					
Staph. aureus	16	6	8.9	2.4	**
Strep. uberis	4	4	2.3	2.0	NS
CNS	41	49	23.0	23.9	NS
Strep. spp.	9	3	3.4	1.0	NS
Strep. agalactiae	4	1	2.6	0.5	NS
Proteus	1	0	0.6	0.0	NS
E. coli	1	0	0.6	0.0	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

Table 7.13 Total numbers of cows sampled, with no infected quarters or infected in at least one quarter; LSmeans of these number (expressed as % of total cows), and the significance of the differences between the two herds in samples fourteen days post-calving

	Number of cows		Lsmeans (% of total cows)		
	Organic	Conventional	Organic	Conventional	Significance
Total cows	45	49			
No growth	10	17	19.7	35.5	NS
Growth of pathogens	35	32	80.3	64.5	NS
Bacterial growth					
Staph. aureus	12	6	27.9	11.5	*
Strep. uberis	4	4	9.1	8.2	NS
CNS	27	28	61.9	55.6	NS
Strep. spp.	9	3	15.8	4.6	*
Strep. agalactiae	4	1	9.1	2.0	NS
Proteus	1	0	2.3	0.0	NS
E. coli	1	0	2.3	0.0	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

The number of quarters showing positive or no growth for each type of bacteria is shown in Table 7.12. Same data for number of cows is shown in Table 7.13. The effect of quarter position was significant for cows with quarters not showing bacterial growth and showing CNS growth. However, no interaction with herd effect was found. The lowest percentage of quarters with no growth was seen in the back quarters of the udder. Coagulase-negative staphylococci showed a similar pattern, where the back quarters presented the highest percentages of growth, as can be seen in Table 7.14.

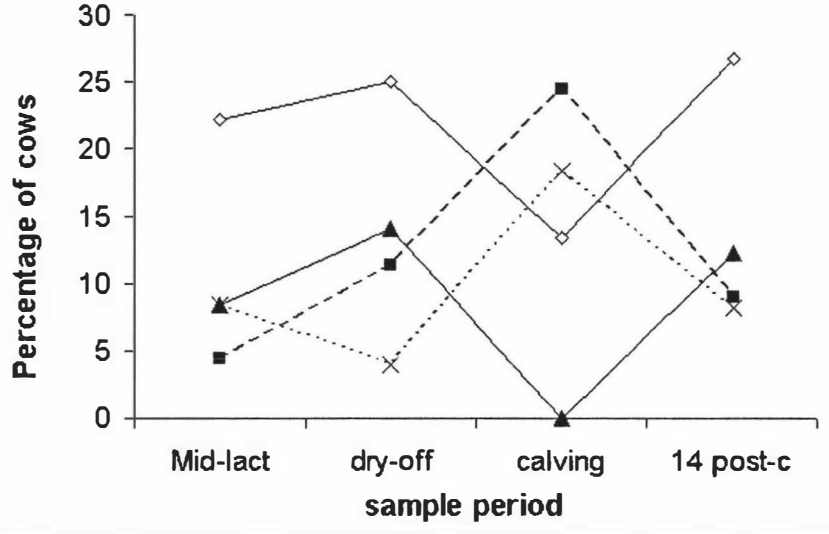
Table 7.14 Lsmeans for no growth and growth of Coagulase-negative staphylococcus (% of total quarters) according to quarter location in samples fourteen days post-calving

	Front right	Front left	Back right	Back left	Significance
No growth	75.4^{a}	82.4^{a}	52.2^{b}	57.1^{b}	****
CNS	19.5^{ac}	15.9^{a}	32.3^{b}	28.8^{bc}	*

Lsmeans with different superscript significantly differ; *= p <0.05, ****= p<0.0001

From all the data presented above, the pattern of bacterial growth shown in cows for both herds by two of the most important bacteria in the New Zealand context, *Staphylococcus aureus* and *Streptococcus uberis*, is shown in Figure 7.1.

Figure 7.1 Pattern for growth of *Staphylococcus aureus* and *Streptococcus uberis* in cows for both herds through the four sampling periods (-▲-Conventional S. aureus) (-◇-Organic S. aureus) (-✕-Conventional S. uberis) (-■-Organic S. uberis)



7.5. Clinical mastitis cases

7.5.1. Season 03-04

During the season 2003-2004, 33 cases of clinical mastitis occurred in the organic herd and 19 in the conventional herd. These cases were seen in 16 animals in the organic herd (nine cows had one clinical case, five cows had two cases, three cows had three cases and two cows had four clinical cases) and 13 in the conventional (eight cows with one case, four with two cases and one cow with three clinical cases). Frequency of mastitis is shown in Table 7.15 and monthly cases in Table 7.16.

Table 7.15 Frequency of cows that presented at least one case of clinical mastitis in each herd in season 03-04 (number of cows)

	Organic	Conventional	Significance
Clinical mastitis	31% (16)	28% (13)	NS

NS= Non significant

Table 7.16 Percentage of animals with clinical mastitis based on total cows in each herd per month in the season 03-04 (number of cows)

	Organic	Conventional	Significance
August	48% (10)	40% (10)	NS
September	71% (17)	22% (6)	**
October	4% (2)	2% (1)	NS
November	6% (3)	2% (1)	NS
December	0% (0)	2% (1)	NS
January	2% (1)	0% (0)	NS

NS= Non significant; *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$

7.5.2. Season 04-05

In the first half of season 2004-05 (August-December 2004) 10 clinical cases were recorded in each herd. These cases were shown in 7 animals in the organic herd (five cows had one clinical case, one cow had two cases and one cow had three clinical cases) and 8 in the conventional herd (seven cows had one clinical case and one cow three cases). Five animals in the organic herd were treated with antibiotics and quarantined as required. Frequency of mastitis is shown in table 7.17 and monthly cases in Table 7.18

Table 7.17 Frequency of cows that presented at least one case of clinical mastitis in each herd in the first half (August-December 04) of season 04-05 (number of cows)

	Organic	Conventional	Significance
Clinical mastitis	16% (7)	17% (8)	NS

NS= Non significant

Table 7.18 Percentage of animals with clinical mastitis based on total cows in each herd per month in the first half (August-December 04) of the season 04-05 (number of cows)

	Organic	Conventional	Significance
August	17% (3)	11% (3)	NS
September	0% (0)	0% (0)	NS
October	17% (7)	10% (5)	NS
November	0% (0)	2% (1)	NS
December	0% (0)	2% (1)	NS

NS= Non significant

7.6. Somatic cell counts (SCC)

7.6.1. Season 2003-2004

Results from the analysis of individual herd tests in the season 2003-04 are shown in Table 7.19

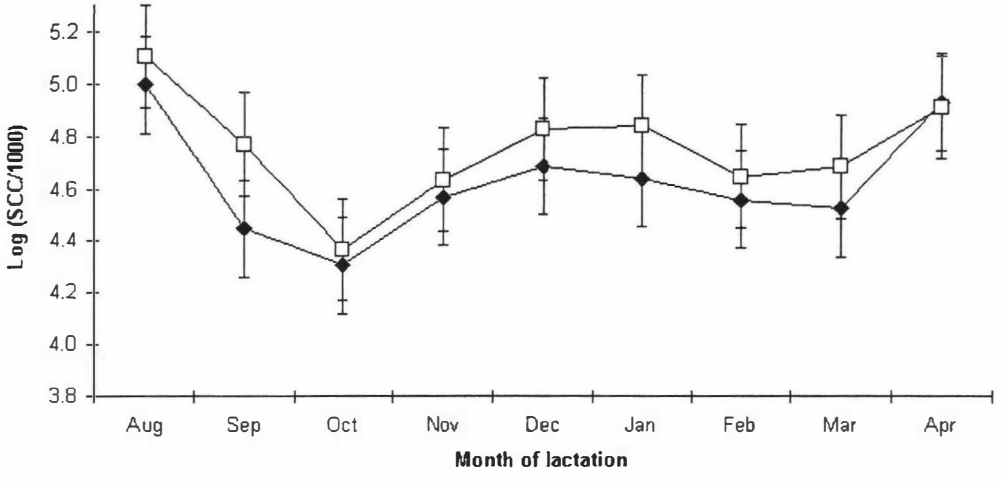
Table 7.19 Lsmeans for somatic cell counts from all individual herd tests in both herds during the season 03-04, natural logarithmic SCC (LSCC), their standard error and significance

	Lsmeans			
	SCC (000 cells/ml)	LSCC	Std error	Significance
Organic	116	4.75	0.1343	NS
Conventional	102	4.63	0.1274	

NS=Non significant

Results for SCC (after a natural logarithmic transformation) on each herd test date were averaged and shown in Figure 7.2. The organic herd generally showed a slightly higher SCC level than the conventional herd but the differences between the two herds were not significant in any month.

Figure 7.2 Monthly average somatic cell counts (log_eSCC) throughout the season 03-04 for both herds (Conventional) (Organic) I= SE

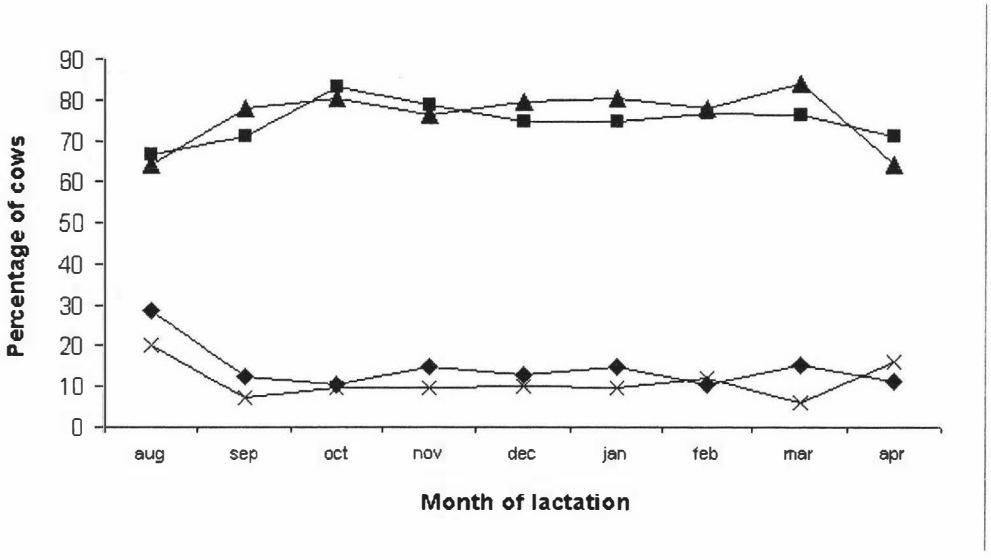


From the herd tests analyzed, the number of animals below 200,000 cells/ml; between 200-400,000 cells/ml and above 400,000 cells/ are shown in Table 7.20, expressed as a percentage of the total number of animals tested per herd at each herd test. The proportions below 200 and above 400 thousand cells/ml are also shown in Figure 7.3 for both herds. There were no consistent differences between the two herds.

Table 7.20 Percentage of cows with Somatic cell counts (SCC) from herd tests below 200, from 200-400 and above 400 (thousand cells/ml) through the season 03-04 in both herds (number of cows)

	<200,000 cells/ml		200-400,000 cells/ml		>400,000 cells/ml	
	Organic	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**
August	67% (14)	64% (16)	5% (1)	16% (4)	29% (6)	20% (5)
September	71% (17)	78% (21)	17% (4)	15% (4)	13% (3)	7% (2)
October	83% (39)	80% (41)	6% (3)	10% (5)	11% (5)	10% (5)
November	79% (37)	76% (39)	6% (3)	14% (7)	15% (7)	10% (5)
December	74% (35)	80% (39)	13% 6)	10% (5)	13% (6)	10% (5)
January	74% (35)	80% (41)	11% (5)	10% (5)	15% (7)	10% (5)
February	77% (36)	78% (39)	13% (6)	10% (5)	11% (5)	12% (6)
March	76% (35)	84% (42)	9% (4)	10% (5)	15% (7)	6% (3)
April	71% (32)	64% (32)	18% (8)	20% (10)	11% (5)	16% (8)

Figure 7.3 Percentage of cows with Somatic cell counts (SCC) with <200 or >400 (thousand cells/ml) for both herds (Organic <200) (Conventional <200) (Organic >400) (Conventional >400), from monthly herd tests in 2003-04



7.6.2. Season 2004-2005

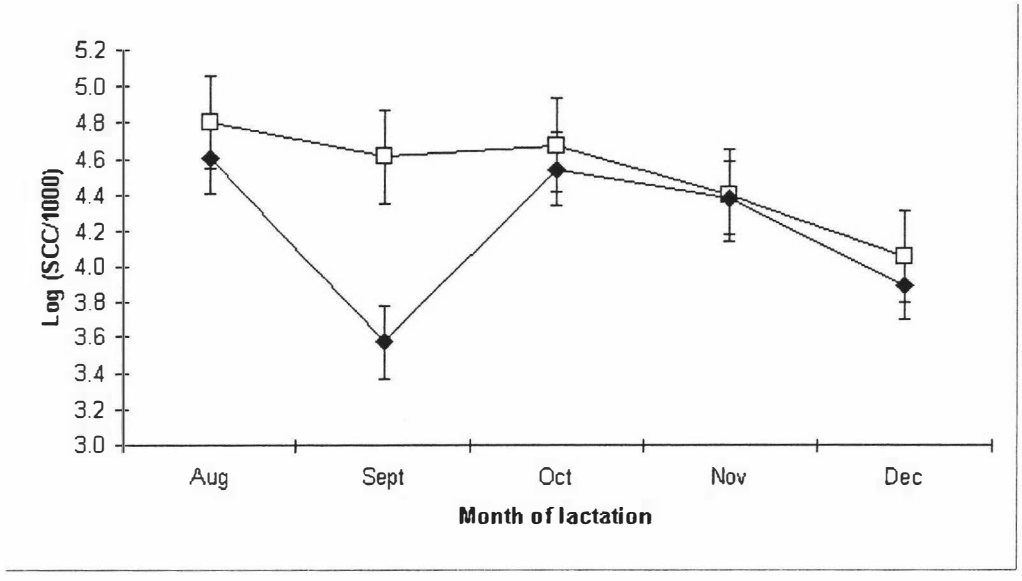
Results for the analysis of SCC from herd tests for the first half of the season 2004-05 (August-December 2004) are shown in Table 7.21, where no significant differences were observed between herds. However, the organic herd showed a slightly higher level of SCC overall, that was just significant in the second month of lactation (P <0.001), as can be seen in Figure 7.4.

Table 7.21 Lsmeans for somatic cell counts (thousand cells/ml) from individual herd tests in both herds through first half of the season 04-05 (August-December 2004), natural logarithmic SCC (LSCC), their standard error and significance

	Lsmeans			
	SCC (000 cells/ ml)	LSCC	Std error	Significance
Organic	91	4.51	0.1515	NS
Conventional	67	4.20	0.1431	

NS= Non significant

Figure 7.4 Monthly average somatic cell counts (log_eSCC) throughout the first part of season 04-05 (August-December 04) for both herds (Conventional) (Organic) I= SE

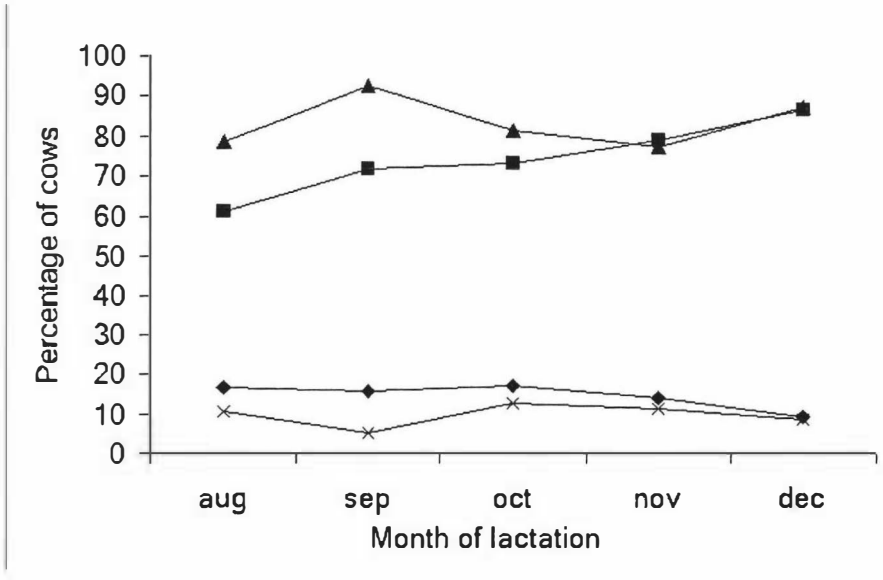


The proportions of animals showing SCC below 200,000 cells/ml; between 200-400,000 cells/ml and above 400,000 cells/ml are shown in Table 7.22, as a percentage of the total number of cows at each herd test. The low (<200, 000 cells/ml) and high SCC animals (>400,000 cells/ml) are also shown in Figure 7.5. The organic herd always showed a slightly higher proportion of high SCC and a lower proportion of low SCC cows compared to the conventional herd.

Table 7.22 Percentage of cows with somatic cell counts (SCC) from herd tests below 200, from 200-400 and above 400 (thousand cells/ml) through first half of the season 04-05 (August-December 2004) in both herds (number of cows)

	<200		200-400		>400	
	Organic	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**
August	61% (11)	79% (22)	22% (4)	11% (3)	17% (3)	11% (3)
September	72% (23)	93% (37)	13% (4)	3% (1)	16% (5)	5% (2)
October	73% (30)	81% (39)	10% (4)	6% (3)	17% (7)	13% (6)
November	79% (34)	77% (34)	7% (3)	11% (5)	14% (6)	11% (5)
December	86% (38)	87% (41)	5% (2)	4% (2)	9% (4)	9% (4)

Figure 7.5 Percentage of cows with somatic cell counts (SCC) with <200 or >400 (thousand cells/ml) for both herds (Organic <200) (Conventional <200) (Organic >400) (Conventional >400), from monthly herd tests for the first half of the season 04-05 (August-December 2004)



Similar proportion of cows with SCC <200 (thousand cells/ml) were shown through the season for both herds. However, the largest difference was shown in early lactation, where the organic herd presented a lower proportion of cows <200 than the conventional herd. In addition, the organic herd showed a higher proportion of cows >400 compared to the conventional herd, as it is shown in Figure 7.5. This is consistent with the data in Figure 7.4.

7.7. Milk solids production

Infected animals, with high SCC, were mostly taken out of the bulk milk tank in the organic herd. Therefore, data for milk solids yield per cow was recorded from monthly herd tests. No significant differences in milk solid yields were shown between herds in both seasons analyzed, although the conventional herd showed a slightly higher yield than the organic herd in both seasons, as seen in Tables 7.23 and 7.24.

7.7.1. Season 03-04

Table 7.23 Lsmeans for milk solids production per cow for each herd for the season 03-04 (kg Milksolids per cow daily)

		Lsmeans		
Herd	**Milk solids**	**LMS**	**Std. error**	**Significance**
Organic	1.76	0.56	0.01	NS
Conventional	1.81	0.59	0.01	

NS= Non significant

7.7.2. Season 04-05

Table 7.24 Lsmeans for milk solids production per cow for each herd for the first half of season 04-05, August-December 2004; (kg Milksolids per cow daily)

		Lsmeans		
Herd	**Milk solids**	**LMS**	**Std. error**	**Significance**
Organic	1.90	0.64	0.02	NS
Conventional	1.98	0.68	0.02	

NS= Non significant

7.8. Dry period treatments, a description of common procedures in each production system

Due to culling of cows at dry-off, and replacement heifers entering the herd at calving (11 animals in each herd), just 34 animals in the organic herd and 36 in the conventional herd remained in the farms for the entire dry period (from dry-off to calving). From those, 15 animals in the organic herd were treated with a teat sealer (Teatseal, Pfizer, Auckland, NZ) after the final milking before dry-off in the season 2003-2004. In the conventional herd, 22 animals were treated with Dry cow therapy; the rest of the animals in both herds received no treatment after dry-off.

Cows were selected for dry period treatments based on their SCC herd test results through the season, and clinical mastitis cases. In the organic herd, animals that showed no clinical cases and had a low somatic cell count at the last herd test (<150,000 cells/ml) were selected for treatment with teat sealer, the rest of the animals were not treated. In the conventional herd, cows were selected for DCT if they showed high SCC

(>150,000 cells/ml) at the last herd test and if they had shown clinical signs of mastitis. If they did not show either of those, animals received no treatment with DCT.

Data was analyzed separately for each of the two herds and treatment group in each herd (DCT, teat sealer or non-treated) and the results obtained at dry-off and at calving were compared to detect changes in the infection status of the animals.

7.8.1. Organic herd

No pathogens were found at calving in any of the animals treated with teat sealer with the exception of CNS. Mostly, animals treated with teat sealer were also free of bacterial growth at dry-off, except for 5.3% of quarters that were positive for CNS and remained infected at calving. In the treated animals just 3.8% of the quarters showed bacterial growth at calving (CNS), compared to 7.5% in the non-treated group. Percentage of "cured" infections was slightly higher in the non-treated group, since fewer animals had positive bacterial growth at dry-off in the treated group, except for *Streptococcus uberis*, where the teat sealer group showed 1.5% compared to 0% in the non-treated group.

Significant differences were shown for growth of *Staphylococcus aureus* and *Streptococcus uberis* between the teat sealer-treated and untreated groups from dry-off. In the group treated with teat sealer, bacterial growth present at dry-off disappeared and no new growth was present at calving, whereas in the non-treated group new infections were present at calving for both pathogens (3% and 9% of quarters for *S. aureus* and *S. uberis* respectively), and bacterial growth present at dry-off was still present at calving (3.8% and 0.8% respectively). However, the percentage of quarters that remained with no bacterial growth was always higher for the non-treated group.

Results from samplings for treated and non-treated quarters in the organic herd at dry-off and calving are shown in Table 7.25

Tabla 7.25 Change of Infection in the organic herd for quarters treated with teat sealer (TS) or not treated (NT) during the dry period; as a percentage of quarters that had new infections, that presented a cured infection and that had no change in infection status (% of total quarters)

	New Infections (%)		Cured infections (%)		No change in infections (%)				Significance of difference
Dry-off: Calving	Not Infected: Infected		Infected: Not Infected		Not Infected: Not infected		Infected: Infected		
	NT	TS	NT	TS	NT	TS	NT	TS	
S. aureus	3.0%	0.0%	0.8%	0.0%	49.6%	42.9%	3.8%	0.0%	*
CNS	7.5%	3.8%	18.0%	17.3%	17.3%	16.5%	14.3%	5.3%	NS
S. uberis	9.0%	0.0%	0.0%	1.5%	47.4%	41.4%	0.8%	0.0%	**
S. agalactiae	0.0%	0.0%	1.5%	0.0%	55.6%	42.9%	0.0%	0.0%	NS
Strep. spp.	3.0%	0.0%	4.5%	3.0%	48.9%	39.8%	0.8%	0.0%	NS

NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

7.8.2. Conventional herd

In the conventional herd, no growth of *Staphylococcus aureus* was shown in any of the groups at calving (DCT or non-treated). Percentages of quarters with new infections at calving were higher in the non-treated group. In addition, the DCT group showed a higher percentage of quarters that remained with no growth from dry-off through calving for all bacteria, and it also showed a higher percentage of "cured" quarters at calving. However, no significant differences were found for changes in frequencies at dry-off and calving of any pathogen with the exception of CNS, where the DCT group showed a significantly higher percentage of "cured" quarters at calving.

Results for treated and non-treated quarters with Dry Cow Therapy (DCT) in the conventional herd at dry-off and calving are shown in Table 7.26

Table 7.26 Change of Infection in the conventional herd for quarters treated with Dry Cow Therapy (DCT) or not treated (NT) during the dry period; as a percentage of quarters that had new infections, that presented a cured infection and that had no change in infection status (% of total quarters)

	New Infections (%)		Cured infections (%)		No change in infections (%)				Significance of difference
Dry-off: Calving	Not Infected: Infected		Infected: Not Infected		Not Infected: Not infected		Infected: Infected		
	NT	DCT	NT	DCT	NT	DCT	NT	DCT	
S. aureus	0.0%	0.0%	0.7%	2.1%	38.0%	59.2%	0.0%	0.0%	NS
CNS	9.2%	7.0%	9.2%	26.8%	9.9%	16.2%	10.6%	11.3%	*
S. uberis	4.9%	3.5%	0.0%	1.4%	33.8%	56.3%	0.0%	0.0%	NS
S. agalactiae	0.0%	0.7%	1.4%	0.0%	37.3%	60.6%	0.0%	0.0%	NS
Strep. spp.	1.4%	0.7%	0.7%	2.1%	36.6%	58.5%	0.0%	0.0%	NS

NS=Non significant; *= p <0.05, **= p<0.01, ***= p<0.001, ****= p<0.0001

Staphylococcus aureus infections in cows older than five years compared to cows from two to five years old. Same results were present at dry-off and 14 days post-calving. This data agrees with the widely reported increased level of infections in older cows. This is probably due to the recognized fact that teat ducts become more dilated with each lactation and this causes a higher risk of pathogens getting into the canal, with a higher level of exposure due to the increased number of milkings (Hogan *et al.*, 1999). However, no significant differences between age groups were found within herds, probably because of relatively small numbers in each age group within each herd.

8.1.2. Mid-lactation samples

At mid-lactation, the percentage of cows showing no bacterial growth was higher than all other sample periods. However, this could have been related to the fact that the samples remained frozen for seven months before culture, whereas samples from the other three periods were cultured immediately after sampling. Despite this, other trials have shown that freezing does not seriously compromise bacteriological results (Murdough *et al.*, 1996), but periods as long as the one presented in this study have not been analyzed previously. Nevertheless, infections have been shown to decrease as lactation advances (Hogan *et al.*, 1999) and this could be the reason for the lower levels of bacterial growth at this sample period.

Of the infected samples, 8% of quarters in the organic herd were positive for *Staphylococcus aureus*, which is similar to data obtained in Switzerland. In that study a prevalence of *S. aureus* of 7.4% was present between days 101-305 of lactation for quarters with a subclinical infection, classified with a California mastitis test (CMT) above 1 (Busato *et al.*, 2000). In the conventional herd, just 2% of the quarters were infected with *S. aureus*, significantly lower than in the organic herd.

Furthermore, the bacteria that showed the highest incidence was CNS for both herds. Similar results were obtained in Switzerland, where CNS has shown previously to be the highest cause of intramammary infections, with 51% found in organic herds between 101 and 305 days of lactation (Busato *et al.*, 2000).

Streptococcal infections showed the lowest prevalence out of all four samplings at the mid-lactation sample. This is probably because *Streptococcus uberis* and *Enterococcus* are predominantly environmental bacteria and occur around calving, decreasing in

8. DISCUSSION

8.1. Bacteriology

8.1.1. General overview

The principal bacteria isolated in all sampling periods were Coagulase-negative staphylococci (CNS), which represented the highest percentage of isolations at dry-off. At calving, the second most commonly isolated bacteria were *Streptococcus uberis*, which has been shown to be an important pathogen around calving in New Zealand conventional herds. Moreover, data from recent research has suggested that CNS and *Streptococcus uberis* are now the most common species detected in infections from New Zealand herds (Pankey et al., 1996, McDougall, 1998).

Staphylococcus aureus showed the lowest percentages of isolation at calving, but increased as lactation progressed. Gram negative pathogens showed a very low prevalence in both herds, and were recorded only in the same animals in two consecutive periods.

No significant differences in bacterial growth were found between the two herds with the exception of *Staphylococcus aureus* in all four sampling periods for quarters, and in all periods except at dry-off for cows. In addition, there were significant differences for infections in quarters caused by *Streptococcus* spp. 14 days post-calving. In all those sample periods, the organic herd showed a higher level of infection than the conventional herd.

The effect of quarter location was significant only in samples from later lactation (mid-lactation and dry-off samples). The highest prevalence of infections at both times was located in the rear quarters, and this difference was significant, when all data was combined across herds. However, the difference was not significant within each herd, probably due to the number of animals in each group.

In addition, in mid-lactation, cows above five years old showed a higher level of infection than younger animals for both herds. This effect was especially marked for

incidence as lactation advances (Hogan *et al.*, 1999). Moreover, *Streptococcus dysgalactiae* infections have decreased in recent years and *Streptococcus agalactiae* have generally become very infrequent in New Zealand, as a consequence of effective mastitis control strategies (Woolford, 1997).

8.1.3. Dry-off samples

Samples at dry-off showed the highest bacterial growth out of all the sampling periods In addition, they presented the highest level of CNS, with 55% of infected quarters in the organic herd and 62% in the conventional herd. Research in those pathogens, considered to be of minor importance, has shown that infections caused by them increase as lactation advances and reach the highest levels at dry off (Timms and Schultz, 1986). Data reported from Wisconsin herds showed that 55% of cows were infected with CNS at dry-off, which is similar to the results obtained in both herds in the present study (Timms and Schultz, 1986). Results from New Zealand conventional heifers at this period showed only 16% of animals infected with CNS, with a range of 3.6-26.5% (Pankey *et al.*, 1996), much lower than in the present study.

At a cow level there were no significant differences in bacterial growth between herds. At a quarter level the organic herd showed a significant higher prevalence of *Staphylococcus aureus*, with 10% of quarters positive for that bacteria, compared to 3% in the conventional herd. The latter is similar to the 2.8% obtained at dry-off in a trial with primiparous heifers from eleven conventional herds in New Zealand, and both of the present herds were in the range (0-11.4%) obtained in that study (Pankey *et al.*, 1996). Moreover, data from 528 cows with low somatic cell counts from three conventional herds in New Zealand showed 9% of quarters infected with *Staphylococcus aureus* (Woolford *et al.*, 1998), similar to the percentage presented in the organic herd in this study.

No significant difference was recorded between herds in cows showing growth of *Streptococcus uberis*. However, there was a higher prevalence in the organic herd, with 11% of the cows with at least one positive quarter compared to 3% in the conventional herd. The latter is similar to 2.8% of heifers infected in 11 conventional New Zealand herds at dry-off (Pankey *et al.*, 1996).

The percentage of quarters with growth of *Streptococcus agalactiae* was low in both herds, with a slightly higher percentage in the conventional herd (0.57% in the organic herd and 1.01% in the conventional herd). However those small differences were not significant. Data from organic herds in Switzerland (Busato *et al.*, 2000) showed 0.8% of quarters infected with that pathogen in late lactation (101- 205 days of lactation), similar to the results for both herds in the present study.

8.1.4. Calving samples

Results at calving showed the largest difference between herds of all the sampling periods, since no cases of *Staphylococcus aureus* were found in the conventional herd whereas 13% of the cows in the organic herd were positive in at least one quarter for this pathogen. However, both herds showed a similar percentage of quarters with no bacterial growth, with the organic herd showing a slightly lower percentage of infected cows compared to those infected in the conventional herd.

Nevertheless, despite the difference of *Staphylococcus aureus* infections between herds, both herds showed in this sample the lowest percentage of animals infected with this pathogen from all samples. Animals positive for this pathogen increased as lactation advanced, in agreement with results for conventional herds in New Zealand, where the prevalence of *Staphylococcus aureus* has been shown to increase after mid lactation reaching higher levels in dry-off (Pankey *et al.*, 1996). Similar results have also been reported for organic herds in Denmark and in UK (Vaarst and Enevoldsen, 1997, Berry and Hillerton, 2002b).

In contrast, the highest percentage for growth of *Streptococcus uberis* was recorded in both herds around calving, in agreement with results for conventional herds in New Zealand (Pankey et al., 1996, McDougall, 1998). However, it was only the second most frequently isolated pathogen at calving, after CNS, with no significant differences between herds.

Coagulase-negative staphylococcus growth was at it highest level at calving, with 76% and 81% of cows positive for them in at least one quarter in the organic and the conventional herd respectively. Data for heifers from 11 herds in New Zealand showed 22% of animals infected, with a range among farms of 4.3-44.8 (Pankey *et al.*, 1996), which is much lower than the values from the present study.

There was no growth of *Streptococcus agalactiae* in the organic herd at this sampling period and just 2% was shown in the conventional herd. However, four quarters in the organic herd and one in the conventional herd were positive for Gram negative bacteria. *Proteus* infections occurred in two animals in the organic herd, one of which had a difficult calving and remained lying down in a stream, causing a general septicemia and clinical mastitis due to *Proteus* in two quarters. Another animal was positive for it in one quarter and showed clinical mastitis. In the conventional herd, one animal was positive in one quarter, and showed generally bad health.

Escherichia coli was present in one quarter of an animal in the organic herd. This particular animal presented a bad udder conformation that made the milking process difficult, since all four teat cups could not be attached at the same time, making it necessary to milk two quarters first and two afterwards. Of those, the most difficult quarter to milk was positive for *E. coli*. Data from Danish organic herds also showed a low incidence of *E. coli* infections, mainly in just one quarter (Vaarst and Enevoldsen, 1997), which agrees with the organic herd in the present study, where just one quarter was infected with *E. coli*, at two sampling periods, despite treatment with antibiotics.

8.1.5. Fourteen days post-calving samples

At this period, growth of *Staphylococcus aureus* was again present in the conventional herd. Nevertheless, there was a significant difference between herds, with the organic herd showing a higher percentage of growth for this pathogen than the conventional herd (28% and 12% of cows respectively).

Coagulase-negative staphylococcus showed the highest growth percentage from all bacteria as in the other sampling periods. Both herds showed 23% of positive quarters (62% of cows in the organic herd and 56% in the conventional herd). High percentages for growth of CNS have been shown previously, such as the 52% of infected quarters (with a CMT above 1) present between days 7 to 100 of lactation in organic herds from Switzerland (Busato *et al.*, 2000), which is higher than the values for both herds in the present study.

Growth of *Streptococcus agalactiae* was low for both herds and was present in just one quarter of all cows, in both herds. Positive growth was slightly higher in the organic herd, compared to the conventional herd; still this difference was not significant.

Nevertheless, growth of *Streptococcus* spp. in the organic herd showed a significantly higher prevalence than in the conventional herd, with 16% of the cows positive in at least one quarter compared to 5%, respectively.

Changes in bacterial growth from calving to 14 days post-calving presented the same effects in both herds. There was an increment in positive cases by *Staphylococcus aureus* and a decrement in growth by any other bacteria. In general, bacterial growth decreased, since there was a lower percentage of positive growth in quarters 14 days post-calving than at calving for both herds.

8.2. Somatic cell counts

Only individual SCC monthly herd test data was used. Bulk milk SCC data was not used since milk from high SCC quarters or cows was withheld in the organic herd.

Data from the season 2003-04 showed a slightly higher somatic cell count in the organic herd, with 116,000 cells/ml compared to 102,000 cells/ml average for all cows at all tests in the conventional herd.

Although the results from the present study found no significant differences between the two herds in the SCC of individual cows, these were slightly higher in the organic herd, which agrees with the analysis of the first season (2001-02) of the present trial (Lopez-Villalobos *et al.*, 2003). Nevertheless SCC results for the season 2003-04 were higher than those present at the beginning of the trial (85 and 71 thousand cells/ml for the organic and conventional herd respectively).

However, both herds were below the average mean of 220,000 cells/ml reported for the 2003-04 season for 74% of the total herds in New Zealand which were herd tested (LIC, 2004). Moreover, results for both herds were below the SCC records from 11 herds converting, or recently converted to organic in England and Wales, that showed mean somatic cell counts of 270,700 and 299,100 cells/ml during the first and second year of organic production (Weller and Cooper, 1996). The mean somatic cell count of 116,000 cells/ml presented by the organic herd in the present study was also lower than the

244,070 reported for one organic herd over a six year period in UK (Weller and Davies, 1998).

Data from Norwegian organic herds showed no differences between organic and conventional herds (Hardeng and Edge, 2001). However, for cows in their second lactation, SCC records in the organic herds were lower than in the conventional herds, whereas for cows in their sixth lactation or later lactations, a higher SCC was recorded in the organic herds. Overall geometric mean SCC was significantly lower (73,700) for the organic herds than for the conventional herds (79,000 cells/ml). This result differs from the present study, where both herds showed similar somatic cell counts.

Data from the first half of the season 2004-05 also showed no significant differences between the herds, although the organic herd again showed a slightly higher SCC level than the conventional herd. The only significant difference was detected in the second month of lactation, when the organic herd presented a higher SCC ($P<0.005$) than the conventional herd (101,000 vs. 36,000 cells/ml) and shows a consistency with bacteriology results, where the highest percentage of infected cows (28%) with *Staphylococcus aureus* was shown from all four samplings in the organic herd (compared to just a 12% in the conventional). However, that difference did not affect the overall SCC results, which remained similar in both herds.

8.3. Clinical mastitis cases

The percentage of clinical cases in the first two months of lactation in season 03-04 for both herds was higher than the 10% reported by McDougall (1998) for the first 6 weeks of lactation in conventional New Zealand herds. The frequency of clinical cases in season 03-04 in the conventional herd (28%) was higher than the annual 14% shown in 139 conventional herds in New Zealand (McDougall, 2001). The frequency for the organic herd was even higher (31%), however, no significant differences were found between the herds when comparing their frequencies nor when comparing them in each month of lactation.

Data from a survey of 11 organic herds in New Zealand showed a range for percentage of clinical mastitis from 1-14% in the season 02-03 (Thatcher, 2003), which are lower than the data presented by the organic herd in the present study.

However, the frequency of clinical cases in the organic herd (31%) was lower than the 40.5% and 46% reported for the first and second year of analysis for 11 organic herds in the process of conversion or recently converted in England and Wales (Weller and Bowling, 2000).

The only difference between the two herds in season 2003-04 was recorded in the second month of lactation, when the organic herd showed 71% of clinical cases compared to 22% in the conventional herd (based on the number of cows calved at that point in each herd). This was also noticed in the SCC levels of that month, where there was a significant difference between herds, with the organic herd showing 118,000 and the conventional 86,000 cells/ml.

No significant differences in clinical cases were found in the first half of the season 2004-05. In the second month of lactation, even though there was a high percentage of cows with a high SCC in the organic herd, no clinical signs were shown in any of these cows, nor in the conventional herd.

Weller and Bowling (2000) showed that the incidence of clinical mastitis in UK organic herds (34%) was similar to that presented by conventional herds (37%). Results were just slightly higher than in the present study and agree in the lack of difference between systems.

However, the frequency of clinical cases in the organic herd was higher than the 19% average reported in an organic herd for a six year period in UK (Weller and Davies, 1998). Cows showing a high somatic cell count in the latter herd were positive for *Staphylococcus aureus*, which agrees with the results obtained in the present study, where 74% and 52% of the high SCC (>400,000 cells/ml) records were from animals infected by *Staphylococcus aureus* for the organic and the conventional herds respectively. Supporting data from previous studies have shown that herds with low incidences of clinical mastitis and high levels of somatic cell counts are more likely to

be infected with contagious pathogens rather than environmental pathogens (Erskine *et al.*, 1988).

Elimination of *Staphylococcus aureus* infections is difficult even with the use of antibiotics in conventional herds (Kerro Dego *et al.*, 2002). Failure to control this pathogenic organism in organic herds could allow it to spread across the herd. Effective preventive and control measures, to avoid the spread of contagious pathogens are essential in all herds, but especially under organic conditions.

Dry cow therapy has shown cure rates of 40 to 70% for *Staphylococcus aureus* and therefore, is considered the best treatment option. However, since this treatment is not allowed in the organic systems, the only possible way of controlling this pathogen and remain free of it is by trying to prevent it and where that fails, by culling all infected animals from the farm (Sears and McCarthy, 2003b).

Data from a monitored conventional herd in United Kingdom showed a decrease in the percentage of quarters infected with *Staphylococcus aureus* from 4.9% to 0.3% over a period of five years by encouraging the five point plan which used antibiotics during lactation and at dry-off (Hillerton *et al.*, 1995).

The present data and from the season 2001-02 (Lopez-Villalobos *et al.*, 2003) suggest that the frequency of clinical and subclinical mastitis has probably increased over the two year period, from 16% to 31% and from 14% to 28% for the organic and conventional herds respectively. These increments could be related to an increment in *Staphylococcus aureus* infections. Nevertheless, no bacteriology was performed in the 2001-2002 season.

Since early lactation in season 03-04, the organic herd presented problems related to mastitis carried by contagious pathogens, where the bulk milk somatic cell count was usually higher than the conventional herd. Bacteriology results confirmed the causative agent when a high proportion of cows infected with *Staphylococcus aureus* were detected. Culling after dry-off at the end of season 03-04 was based mainly on animals infected with this pathogen in all quarters. However, not all infected animals could be culled due to the low number of replacement heifers.

Cows infected with *Staphylococcus aureus* in the present study were then segregated and identified at the beginning of lactation in season 2004-2005. Infected animals were milked after the uninfected cows, and quarter milkers were used to prevent high somatic cell counts in the milk tank, which meant a labor intensive process during milking. Finally, in mid lactation 04-05, infected quarters were dried-off and not milked any longer which facilitated the milking process, and probably reduced the risk of further infections.

In the conventional herd, culling was based on empty animals and low producers. At the beginning of season 04-05, animals known to be infected with *Staphylococcus aureus* were not segregated during milking since the bulk tank milk did not reach such high levels (near penalty) as the organic did. Therefore, those infected animals were just regularly checked and considered for culling after dry-off of the current season.

Teat condition has shown to be an important factor to reduce the risk of contamination, especially related to contagious pathogens, since they have a limited chance of surviving on the skin if it is healthy. Therefore, correct teat disinfection after milking, a good level of moisture (>10%), correct functioning of the milking machines and avoidance of over-milking are important factors to limit teat chapping (Zecconi and Smith, 2003).

8.4. Dry period treatments; descriptive analysis

Due to normal, commercial management procedures in each herd, the selection of animals for treatment at dry-off was done according to their previous SCC and their clinical records. As a consequence, the treatment groups were very biased. In the organic herd, animals in the "teat sealer group" had very low somatic cell counts and no previous clinical cases, whereas the non-treated animals had high SCC, and some had previously shown clinical mastitis cases. In the conventional herd, animals treated with DCT presented high somatic cell counts and/or clinical mastitis before dry-off, whereas the non-treated group consisted of animals with low SCC and no clinical mastitis before drying off. Therefore analysis of these data was simply descriptive since "teat sealer"

was used mainly in "uninfected" cows, but not in "infected" cows, and vice versa for DCT.

8.4.1. Organic herd and teat sealer.

In the organic group, there were no significant differences between animals treated or non-treated with teat sealer except for *Staphylococcus aureus* and *Streptococcus uberis* infections, where the non-treated group had always higher percentages of infection that the treated group. However, the percentage of animals that remained uninfected at dry-off and calving was slightly higher in the non-treated group for all bacteria. No infections were detected at calving for the treated group with the exception of Coagulase-negative staphylococci. For the non-treated group new infections at calving were caused by all bacteria except *S. agalactiae.*

Results from a trial with organic and conventional cows in UK showed less new infections in cows treated with teat sealer compared to a negative control (Berry and Hillerton, 2002a). The non-treated group in the present study showed similar percentages as the UK trial for new quarters positive for *Staphylococcus aureus* but less positive for *Streptococcus uberis.* However, in the present study the treated group presented no new infections for any major pathogen.

Data from New Zealand conventional herds showed significant differences in *Streptococcus uberis* infections, where cows treated with teat sealer have shown lower percentages of new infections than non-treated controls (Woolford *et al.*, 1998). Data from the present study agrees with those results, showing lower percentages of infection for the group with teat sealer for *Streptococcus uberis* infections compared to the non-treated group. *Staphylococcus aureus* infections were also higher in the non-treated group (no cases were present in the treated group). However, number of quarters analyzed in the present study was very low and it was not possible to perform a full analysis of these data.

The percentage of "cured" infections in the non-treated group was higher than the treated group; nevertheless, the first group had also more infections at dry-off. However, in both groups, the highest percentage of "cured" infections was for CNS, followed by *Streptococcus* spp. Data from 16 UK herds agreed with the present study, where both studies showed the highest percentage of "cured" quarters for CNS,

followed by *Streptococcus* spp. infections in treated cows. However, a higher percentage of infections was shown in the present study compared to the UK (Huxley *et al.*, 2002).

8.4.2. Conventional herd and dry cow therapy

In the conventional herd, no significant differences were shown between animals treated with Dry Cow Therapy (DCT) and non-treated, except for CNS infections, where the percentage of "cured" infections in the DCT group is considerably higher than the non-treated group. The percentage of quarters that remained not infected from dry-off until calving was always higher for the DCT group than for the non-treated group. No growth of *Staphylococcus aureus* was shown at calving for any of the groups.

Percentage of quarters with new infections was higher in the non-treated group. Data from New Zealand conventional herds showed low percentage of new infections in cows treated with DCT and results were similar to the present study for all bacteria except for CNS, where the present study showed a higher percentage of CNS infections. However, both studies showed low levels of new infections (Woolford *et al.*, 1998)

In general, studies analyzing the effect of DCT have shown a higher percentage of cured infections at calving than in non-treated quarters, which was the case in the present study, where the highest percentage of cured infections was for CNS, then for *Staphylococcus aureus*, *Streptococcus* spp. and *Streptococcus uberis*.

9. CONCLUSIONS

No significant differences in bacterial growth were shown between the organic and the conventional herd through the season 2003-2004 and the first half of the season 2004-2005, with the exception of *Staphylococcus aureus*, where the organic herd always showed a higher bacterial growth than the conventional herd. The same significant difference happened in cows positive for *Streptococcus* spp. 14 days post-calving.

In addition, the organic herd showed a slightly higher level of somatic cell counts over both seasons, which was significantly higher only in early lactation of season 04-05. Clinical cases were significantly different between herds only in the early lactation of season 03-04, where the organic herd showed a higher percentage of cases than the conventional herd.

The most common pathogens in both herds were Coagulase-negative staphylococcus (CNS), which are considered minor mastitis pathogens. However *Staphylococcus aureus* showed the main differences between herds. This is a contagious pathogen and was probably the cause for differences in SCC, since high somatic cell counts and low levels of clinical mastitis cases have been related to infections caused by contagious pathogens, which was the case for the organic herd in the present study.

Nevertheless, general infection patterns present for both herds in this study agree with patterns from New Zealand conventional herds and shows that organic herds could experience infection patterns that are similar to those in conventional herds. Significant differences in growth of *Staphylococcus aureus* could be a result of more infected glands in the organic herd serving as reservoirs that helped to spread the bacteria, of a possible failure to cull some chronically infected cows and the lack of opportunity to treat with antibiotics at dry-off or in lactation.

Percentage of cows showing bacterial growth remained similar between herds through all four samplings, which probably shows that the organic herd was not more infected than the conventional herd, but rather that more of the infections were caused by contagious pathogens than in the conventional herd.

For conventional herds it is difficult to eradicate these contagious pathogens, even with the use of antibiotics, and culling is usually the best tool to obtain an infection-free herd. Therefore, organic herds require more intensive labor, where constant check ups of teats and milk are vital. Consequently, organic herds must rely on their preventive processes to avoid clinical cases that will be difficult to treat with non-antibiotic treatments, which will result finally with the culling of infected cows.

In the organic herd of the present study it is intended that the incidence of *Staphylococcus aureus* will be reduced by targeted culling, and by vigorous hygienic means. However, in cases where culling is not an option, due to a low number of replacement heifers for the herd, the only other option will be to milk the infected animals last and keep them under close observation. Also, preventive measures have to be encouraged, since significant differences in *Staphylococcus aureus* infections could be a result of more infected cows spreading the infection through the herd.

During season 2004-2005 there was a lower incidence of clinical cases in early lactation than in the previous season, where a higher percentage of animals showed clinical signs and the somatic cell counts reached high levels. However, a more strict control of infected animals was possible in season 04-05 due to the availability of the bacteriology results at calving and two weeks later, which, together with the somatic cell count data received every month for each animal, allowed constant monitoring of the infected animals and their segregation during milking.

These results show a higher percentage of clinical cases than in season 2001-2002, which could suggest that incidence of infections has increased over the last two years, especially for the organic herd, possibly due to *Staphylococcus aureus* infections, or that detection has vastly improved.

Control of contagious pathogens in organic herds is limited due to the lack of antibiotic treatment. However, strict teat disinfection, correct milking practices and isolation of infected cows could restrict mastitis infections and control its spread in the herd.

Generally in organic herds, mastitis control measures emphasize the prevention of the disease, rather than its treatment, which is usually performed by alternative methods. However, data coming from studies of homeopathic and other alternative treatments to

treat mastitis have not shown any consistent results, partly due to the absence of negative controls in the design of trials. Therefore, severe clinical mastitis cases are still treated with antibiotics when the welfare of sick animals is involved. Despite this, producers who want to comply with the current USDA organic standards will face the fact that these do not allow an animal to be retained if it has received any antibiotic treatment in its lifetime, and treated animals must be removed from the herd. Consequently, the importance given to prevention in organic herds will be highlighted even further in herds which must comply with these USDA organic standards. This will be the case for all organic herds in New Zealand from 2007 onwards.

In organic herds with intramammary infections caused by contagious pathogens, effective control procedures should then be enhanced and made more vigorous. Milk and skin injuries are the most important sources for infections caused by these pathogens; and as a consequence, infected glands become the main source of *Staphylococcus aureus* in these farms. Therefore, animals known to be infected must be isolated by being milked last and by sanitation of the milking machine after the milking of infected cows has being carried out effectively.

However, analyses of further seasons in the herds from the present study should be performed to detect any differences in *Staphylococcus aureus* infections over time, especially in the organic herd, to analyze the effect of the preventive methods in its control.

In general, skilled herd managers should be able to control mastitis in organic herds as in any conventional herds despite the limitations imposed by the inability to use antibiotics. Successful control of mastitis has been shown before in organic herds that enhance prevention by good milking practices.

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11. APPENDIX

11.1. Appendix 1: Age effect on intramammary infections

When analyzing the microbiology data, in most cases, the effect of age did not interact with the effect of herd. Therefore, both herds showed similar tendencies of bacterial growth related to age. However, small numbers of animals of each age group were present for each herd; as a consequence, the effect of age was not significant and is just shown for both herds combined. When the model showed to be valid for the age effect by herd (even if not significant), the results are shown.

In mid-lactation, the effect of age was significant for no bacterial growth and for growth of *S. aureus*, as is shown in Table 11.1, where animals above 5 years showed higher rates of infection than animals below 4 years. However, age did not interact with the herd effect. Therefore, the effect was the same for both herds.

Table 11.1 Lsmeans for bacterial growth (% of cows) from both herds, according to their age group in mid-lactation samples

	2-3 yrs	4-5 yrs	>5 yrs	Significance
No growth	53.1^{a}	31.2^{ab}	14.6^{b}	**
Staph. aureus	5.8^{a}	3.6^{a}	32.8^{b}	**
Strep. uberis	2.4	13.2	5.9	NS
CNS	39.8	63.7	64.9	NS
Strep. spp.	2.3	4.3	3.2	NS

Lsmeans with different superscript significantly differ; NS= Non significant; *= p <0.05, **= p<0.01, ***= p<0.001

Table 11.2 Lsmeans for no growth and growth caused by *S. aureus* and *S. uberis* (expressed as % of cows) for each herd according to their age group in mid-lactation samples

	Organic				Conventional			
	2-3 yrs	4-5 yrs	>5 yrs	Significance	2-3 yrs	4-5	>5 yrs	Significance
No growth	47.6	18.2	7.7	NS	57.9	45.5	22.2	NS
Staph. aureus	14.8	44.5	50.0	NS	4.0	27.3	15.4	NS
Strep. uberis	3.7	22.2	12.5	NS	8.0	0.0	15.4	NS

NS= Non significant

Lsmeans for each herd are shown just for growth of *S. aureus* and *S. uberis* and for no bacterial growth in Table 11.2.

At dry-off, same tendencies were shown. Age showed a significant effect only for growth of *S. aureus*, but had no interaction with the herd effect. Consequently, both herds showed a similar tendency of bacterial growth according to age groups as shown in Table 11.3 (the model could not be fitted for no bacterial growth and some bacteria due to the small numbers of animals in some classes).

Table 11.3 Lsmeans for bacterial growth (% of cows) from both herds according to their age group in dry-off samples

	2-3 yrs	4-5 yrs	>5 yrs	Significance
Staph. aureus	6.9^{a}	17.1^{ab}	35.7^{b}	**
Strep. uberis	2.1	7.9	12.2	NS
CNS	87.5	95.5	93.7	NS

Lsmeans with different superscript significantly differ; NS= Non significant; *= $p < 0.05$, **= $p < 0.01$

Lsmeans for growth of *S. aureus* for each herd according to their age group is shown in Table 11.4.

Table 11.4 Lsmeans for growth of *S. aureus* (% of cows) for each herd according to their age group in dry-off samples

	Organic				Conventional			
	2-3 yrs	4-5 yrs	>5 yrs	Significance	2-3 yrs	4-5	>5 yrs	Significance
Staph. aureus	10.0	27.3	46.1	NS	5.0	9.1	26.3	NS

NS= Non significant

At calving, no significant effect was shown according to age groups. Still, a higher significant percentage of bacterial growth was shown in cows above five years as seen in Table 11.5. Lsmeans in each herd for cows with no growth and infected with *S. uberis* are shown in Table 11.6.

Table 11.5 Lsmeans for bacterial growth (% of cows) from both herds according to their age group in calving samples

	2-3 yrs	4-5 yrs	>5 yrs	Significance
No growth	19.5	34.5	7.3	*
Strep. uberis	19.6	11.0	33.6	NS
CNS	75.2	65.4	89.1	NS
Strep spp.	9.8	11.4	14.6	NS

NS= Non significant

Table 11.6 Lsmeans for bacterial growth caused by *S. uberis* and no bacterial growth (% of cows) for each herd according to their age group in calving samples

	Organic				Conventional			
	2-3 yrs	4-5 yrs	>5 yrs	Significance	2-3 yrs	4-5	>5	Significance
No growth	23.8	38.5	9.1	NS	15.8	30.8	5.9	NS
S. uberis	14.3	15.4	54.5	NS	26.3	7.7	17.6	NS

NS= Non significant

At 14 days post-calving, age showed a significant effect for growth of *S. aureus* but had no interaction with herd effect. Cows below four years showed a lower prevalence than older animals, as seen in Table 11.7. Lsmeans for no bacterial growth and growth of *S. aureus* according to age group are shown in Table 11.8.

Table 11.7 Lsmeans for bacterial growth (% of cows) from both herds, according to their age group in samples fourteen days post-calving

	2-3 yrs	4-5 yrs	>5 yrs	Significance
No growth	18.1	56.1	14.9	NS
Staph. aureus	8.8^{a}	16.0^{ab}	38.0^{b}	*
CNS	62.4	62.4	75.6	NS
Strep spp.	17.4	3.4	10.4	NS
Strep. agalactiae	2.8	8.5	4.3	NS

Lsmeans with different superscript significantly differ; NS= Non significant; *= $p < 0.05$

Table 11.8 Lsmeans for no bacterial growth and bacterial growth caused by *S. aureus* (% of cows) from both herds according to their age group in samples fourteen days post-calving

	Organic				Conventional			
	2-3 yrs	4-5 yrs	>5 yrs	Significance	2-3 yrs	4-5 yrs	>5 yrs	Significance
No growth	9.5	58.3	9.1	NS	31.6	53.8	23.5	NS
S. aureus	16.7	63.6	5.3	NS	15.4	17.6	50.0	NS

NS= Non significant