THE EPIDEMIOLOGY OF MASTITIS IN AUSTRALIAN DAIRY CATTLE

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Abstract

This study represents an aggregation of knowledge on mastitis within the Australian dairy industry. Aspects of the epidemiology and economics of mastitis have been collated and areas of missing knowledge identified. A clinical treatment trial was conducted on subclinical mastitis to identify the role of therapy upon subclinical infection. The effect of individual variables on mastitis risk was studied and aggregated in order to facilitate the development of a computer simulation model of mastitis within Australian dairy herds.

A literature review of mastitis within the Australian dairy industry was conducted. The economic impact of mastitis was examined and the pathway of economic loss to the dairy industry is discussed. The epidemiology of mastitis was studied with special emphasis on quantification of the effect of individual risk factors on the occurrence of disease. Performance parameters for the current diagnostic tests applied within the dairy industry are presented and their suitability for use in a commercial environment discussed. The impact of self-cure and the efficacy of therapeutic intervention in the disease are examined. The role of culling is presented. The chapter concludes with an estimation of the total economic losses experienced on a commercial dairy farm in Victoria in 1998 for three different mastitis levels. The economic benefit to be gained from a reduction in mastitis is also presented.

A clinical treatment trial of subclinically infected cows (high somatic cell count) was conducted in order to determine if therapeutic intervention was an effective management tool. Cows with somatic cell counts in excess of 500,000 cells per ml and more than 14 days calved were selected and randomly assigned to treatment and control groups. A pooled quarter milk sample was taken prior to treatment and repeated at around six weeks after treatment. Treated cows received a course of intramammary and parenteral antibiotics and control cows were untreated. Cows were followed for the rest of the lactation of treatment and into the subsequent lactation and somatic cell counts were recorded. The major pathogens identified were *S aureus* and *S uberis*. Treatment did not have a significant or commercially useful effect upon bacteriological cure rates, survival of cows to the next lactation or somatic cell count for the remainder of the lactation. Treatment of high somatic cell count cows during lactation is not recommended and is discussed.

A requirement exists for the development of a stochastic simulation model of mastitis within Australian dairy herds. The structure of such a model was developed and is presented. Underlying production and somatic cell count responses in Australian cattle were derived. Infection status variables were included and stochasticity was introduced through the use of control variates. State transition probabilities were collected from the literature. Deficiencies in knowledge were identified and methods for modelling these deficient areas discussed. The aggregated information is presented. It is expected that a working stochastic simulation model of mastitis within Australian dairy herds will be developed from information collected in this dissertation.

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List of abbreviations

ABV	Australian Breeding Value
AMMTA	Australian Milking Machine Trade Association
BMCC	Bulk milk cell count
CMT	California mastitis test
Coag. neg. Staph	Coagulase negative staphylococcus spp.
DCT	Dry cow therapy
DOF	Degrees of freedom
DRDC	Dairy Research & Development Corporation
E. coli	Escherichia coli
EBMSCC	Estimated bulk milk somatic cell count
EC	Electrical conductivity
GMSCC	Geometric mean somatic cell count
ICCC	Individual cow cell count
IQCC	Individual quarter cell count
LT	Lactation therapy
MHI	Maffra Herd Improvement Co-operative Ltd.
MID	Macalister Irrigation District
NAGase	N-acetyl-α-D-glucosaminidase
NIR	New infection rate
PI	Production index
QIR	Quarter infection rate
S. aurues	Staphylococcus aurues
S. uberis	Streptococcus uberis
SAMM	Seasonal Approach To Managing Mastitis
SCC	Somatic cell count
SCS	Somatic cell score
SOL	Stage of lactation (days)
VMRG	Victorian Mastitis Research Group

Introduction

Mastitis is a major disease of dairy cattle. Financial losses due to mastitis are substantial and arise in a number of ways including reduced milk yield, reduced milk quality, and increased culling rates, treatment costs and prevention costs. Studies have indicated that mastitis is the most expensive disease on dairy farms in terms of disease cost and expenditure on prevention.

The current world trend towards the elimination of domestic tariffs has had major implications on trade. Reduced protectionism has resulted in increased use of non-tariff trade barriers to protect markets with quality standards of supplied product assuming greater importance. Mastitis, and especially subclinical mastitis, is a major cause of reduced product quality in the dairy industry. This effect is predominantly mediated through increased somatic cell counts (SCC) in milk. Failure to control mastitis can be expected to result in reduced prices for dairy products and loss of export markets. The economic effects of mastitis on major dairy product exporting countries like Australia and New Zealand is likely to increase in the future.

The increasing importance of product quality implies that the traditional method of treating subclinically infected cows at the end of lactation is likely to become inadequate in the near future. Demand exists for economical treatments for subclinical mastitis during lactation that result in significant improvements in milk quality for the remainder of the lactation.

This dissertation represents a collection of information on the epidemiology of and economics of mastitis within Australia. Areas of deficient knowledge are identified and a clinical trial to investigate one specific area of deficiency was undertaken (a clinical treatment trial of high SCC cows during lactation). The accumulated knowledge was presented in a form that would allow development of a computer simulation model of the economics of mastitis in Australian dairy herds. The development of such a model was considered important in order to allow the importance of individual risk factors to be estimated, to identify critical control points within the Australian dairying system, to monitor the success of mastitis control programs and to identify areas requiring further research.

Chapter 1

Review of subclinical mastitis

Introduction

Mastitis refers to inflammation of the mammary gland characterised by physical, chemical and usually bacteriological changes in milk (Blood et al., 1989). The majority of infected glands show no visible changes and produce milk with no gross abnormalities. These infections are termed subclinical mastitis. This review is a concise summary of mastitis, with special emphasis on subclinical mastitis.

Economics of subclinical mastitis in Australia

Various workers have estimated the economic effects of mastitis. A survey of commercial dairy farms in the U.S.A. (Sischo et al., 1990) indicated that clinical mastitis was the most expensive disease on dairy farms, accounting for 24% of all disease costs, and that expenditure on mastitis prevention represented 75% of all farm disease prevention costs. Recent (1997) New Zealand work (Steffert, pers. comm.) indicated that mastitis costs the average New Zealand dairy farm in excess of $14,000 per year. One Australian study conducted in 1982 by the Victorian Mastitis Research Group (VMRG) estimated that each clinical case of mastitis resulted in average losses of $121 per case and that each subclinical case produced an average of $31 less milk than uninfected cows (1980 prices). An investigation of the effect of subclinical mastitis on herd milk fat Production Index (PI) indicated that subclinical mastitis resulted in a lowering of PI by 3-5 points. This equates to a loss of production of 3-5% when compared to herd mates (Youl, B.S.).

A review of economic analyses of mastitis (Schepers et al., 1991) demonstrated large variation between estimates of financial loss due to mastitis. The major reason given for large variation between studies was differences in the analytical techniques employed. Most studies did not include all causes of loss.

Financial losses due to subclinical mastitis arise in a number of ways, but have been categorised as less efficient production and increased veterinary costs, reduced sale value

and idle production factors, and lost future income due to premature disposal (Schepers et al., 1991). A more complete investigation should include prevention costs (McInerny et al., 1992). It is difficult to fully satisfy all criteria, as many costs are unable to be partitioned to a particular disease in the Australian and New Zealand farming systems. For example, milking machine maintenance costs cannot be fully assigned to mastitis prevention as milking machines must be serviced to ensure complete milk harvesting and fast milking times are maintained. Similarly, reduced feed intake by mastitic cows is likely to result in increased feed intake by herd mates in the pasture based Australian and New Zealand dairy industry. Some aspects of treatment (eg dry cow therapy and culling) have disease prevention advantages through reduction in herd prevalence of disease. As such, only direct losses due to mastitis will be examined.

Loss of production

Estimates of loss of production due to subclinical mastitis are highly variable in the literature. Quarter yield loss estimates reported range from 9.0% to 43.3% (Dohoo et al., 1982). An Australian study estimated lactation yield losses to average 80 litres of milk (2%) for every doubling of SCC (VMRG, 1982). The average SCC of infected cows in this study was 3.8 times higher than uninfected cows. This equated to an average loss of 154 litres of milk for each subclinically infected cow.

Some researchers have interpreted the relationship between SCC and yield loss to be linear; others consider the relationship to be curvilinear (Raubertas et al., 1981; VMRG, 1982; Munro et al., 1984; Dohoo et al., 1984 etc.). Most quarter yield studies have involved comparisons within cows. The method employed to cater for compensatory increase in production in uninfected quarters is the major reason for variation in published estimates. Some researchers consider the evidence for compensatory production increase in uninfected quarters to be insignificant (Hoare, 1982), however recent work involving identical twins from New Zealand has demonstrated that significant compensation occurs (Woolford et al., 1983). The findings from this study indicate that yield loss in subclinically infected quarters when compared to uninfected quarters is 20.4% for cows and 36.6% for heifers. However, when uninfected quarters from the infected cow group were compared to identical quarters from the uninfected control group a compensatory yield response was apparent. Uninfected quarters on infected cows increase yield by 13.3%, whereas this increase in heifers was only 2.5%.

A review of studies investigating production losses at the cow level due to subclinical mastitis (Hoare, 1982) had losses ranging from 0-25%. The reasons for such variation between studies include the large number of other variables affecting milk production and the type, severity and duration of infection. For example, herds with poor mastitis control can be expected to have increased numbers of infected cows. Quality of mastitis control may be a surrogate variable for overall farm management. Thus farms with high levels of mastitis may also have low production due to lower quality of management. Bias of this nature will inflate estimates of production loss due to subclinical mastitis.

Management, environmental and genetic bias was controlled in recent NZ work involving monozygotic twins (Woolford et al., 1983; Woolford, 1985). Two sub herds were established, one contained an infected twin and the other contained the uninfected twin. Both sub herds were run together and milked in the same shed thus exposing both groups to identical management. On an animal basis the yield loss due to infection in cows was small and not significant (1.7% less litres and 2.6% less fat), however for heifers significant yield losses occurred (7.8% less litres and fat). These animal losses were similar to estimates derived from the quarter loss data when compensatory yield response and quarter infection rate (QIR) was considered. In the subsequent lactation yield losses were small and insignificant comparing cured cows (based on SCC) and persistently infected cows. All infected heifers were cured at drying off but milk production as three-year-olds was affected by infection status as heifers. Milk production from three-year-olds infected during their first lactation was 5.7% less than from three-year-olds uninfected during their first lactation. Milk fat was reduced by 8.4%. All differences were significant. The prolongation of yield loss into the subsequent lactation following infection of heifers is a major finding of this study.

The pathogen involved does determine the degree of yield loss. In a recent study from the USA minor pathogens were found to produce a 2-3 fold increase in SCC but no significant loss of production (Kirk et al., 1996 b). *Staphylococcus aureus* produces foci of infection in tissues leading to abscess formation. Blockage of glandular ducts and physiological derangement cause premature involution of lobules that supply the duct. Once a lobule has involuted, it does not regain secretory capacity for the remainder of the current lactation (Hoare, 1982). Streptococci produce severe effects in the ducts with no tissue invasion.

Estimates of yield loss at the herd level are also variable. Herd mastitis level, as measured by bulk milk cell count (BMCC), is correlated with herd milk production (Hoare, 1982). Herd level data is also prone to management and environmental bias. For example, in one study losses of 1.4 litres/cow/day were observed between cows for every increase of 1,000,000 cells/ml on composite cow samples. However, the loss between herds was 2.7 litres/cow/day for BMCC increases of 1,000,000 cells/ml (Dohoo et al., 1982). The authors attributed these differences to other management deficiencies that accompany poor mastitis control. Of most relevance to Australia and New Zealand is the identical twin study by Woolford et al. (1983). The uninfected twin group had no infected quarters resulting in an average BMCC for the season of 66,000 cells/ml. This is similar to the lowest herd average BMCC supplied to New Zealand dairy companies. The infected twin group had an average of 1.59 quarters infected per cow resulting in a BMCC for the season of 739,000 cells/ml. Only 2% of farms in New Zealand have a BMCC in excess of 750,000 cells/ml, thus this sub herd is representative of the other extreme of the New Zealand dairy industry. The yield loss in infected compared with uninfected sub herds was estimated at 3.5% for milk and 5.1% for fat. This finding supports field observations. Severe mastitis outbreaks in commercial herds previously enjoying low infection status are not usually associated with dramatic changes in herd milk or fat production. Similarly, elimination of mastitis from infected herds is not usually associated with significant increases in farm production.

Reduction in milk quality

Milk composition changes following infection have been reported (Munro et al., 1984; Hoare, 1982; Dohoo et al., 1982; Lacy-Hulbert et al., 1995). The most apparent change is an increase in blood constituents in milk following infection. This effect is most likely mediated through increased permeability of the secretory epithelium following atrophy or involution of lobules in infected glands (Hoare, 1982). The major blood constituents to increase in milk in subclinically infected quarters are leukocytes, immunoglobulins, serum albumin, α2-macroglobulin, chloride and sodium (Munro et al., 1984). Other ions like magnesium, zinc, iron and copper are also found in slightly higher concentrations. Alterations to the ion concentrations within milk following infection affect milk electrical conductivity (EC) and pH. The pH of milk increases with increasing mastitis infection. Changes in EC and pH have been used as diagnostic tests for subclinical mastitis (Woolford et al., 1982).

The increase in leukocytes is the major cause of increases in SCC in milk. High SCC milk has reduced shelf life and a decreased flavour score, even following pasteurisation (Munro et al., 1984).

Total solids and solids non-fat decrease in milk from mastitic quarters. The effects upon fat levels are not as great but there appears to be a lowered fat content when cell counts increase markedly. Milk fat composition changes are more apparent. Phospholipid concentration decreases and cholesterol concentration increases resulting in a reduced amount of fat globule membrane material present in mastitis milk. This causes longer churning times and weaker bodied butter and cream (Munro et al., 1984). There is a decrease in saturated fatty acids and an increase in saturated fatty acids. More free fatty acids are found in high SCC milk. This appears to contribute to rancidity in milk products (Munro et al., 1984).

Total protein changes in mastitic milk are variable and reflect changes in component parts. Casein levels decline with increasing SCC and individual casein components (α_3, β, κ, and λ) are found in different amounts compared to milk from uninfected quarters. β-Lactoglobulin and α-lactalbumin levels decline and α_2-Macroglobulin levels increase as SCC increases. These changes have detrimental effects on manufacturing properties (Munro et al., 1984). Cheese yields are dependent upon the casein levels in milk and cheese yield losses due to mastitis are regarded as substantial (Dohoo et al., 1982). Decreased pH of mastitis milk and increased presence of α_2-macroglobulin results in longer rennet clotting times and poor curd characteristics. The end result is increased processing cost with decreased cheese quality (Munro et al., 1984).

The levels of many enzymes in milk increase as a result of mastitis. Elevated levels of lipase, protease and some oxidases produce fat and protein stability problems resulting in flavour defects and reduced shelf life (Munro et al., 1984). Mastitic milk is associated with increased levels of inhibitor factors. These inhibitors are suspected to be antibodies against the causative organisms of mastitis. Some are heat stable and thus can impede some culture starters, impacting negatively on the production of certain yoghurts.

Risk of clinical episodes

There is little published data on the probability of clinical cases of mastitis arising from subclinical infections. Studies have demonstrated that up to 50% of subclinically infected

cows, as determined by the California Mastitis Test (CMT), may experience a clinical episode during lactation (Hoare et al., 1977). In this study, reduction in number of quarters infected resulted in a proportional reduction in number of clinical cases. Canadian case-control studies have demonstrated that cows with high SCC are more likely to experience a mild clinical episode than cows with low SCC (Dohoo et al., 1984). Other Australian work has estimated the risk of clinical episodes in high SCC cows to be between 20 and 50% (VMRG, 1982). This study estimated the financial losses for a clinical case to be $31 (1980 prices). The present figure is likely to be $75 per case when adjustments for inflation are made.

Wide variation of incidence of clinical cases between herds with similar BMCC has been observed. In one study examining low BMCC, the clinical case incidence varied from 2.2 to 53.6 cases per 100 cows per year (Schukken et al., 1989). The relationship between number of quarters subclinically infected and number of clinical episodes of disease only exists for contagious pathogens (Hillerton et al., 1995). This study demonstrated that a reduction in number of cows and quarters infected by major pathogens was not associated with a reduction in number of clinical cases as the predominant cause of clinical mastitis was environmental mastitis pathogens (65% of all clinical cases). However, the number of clinical cases due to coagulase positive staphylococci was reduced.

It has also been observed that increased clinical cases are associated with low BMCC, a low prevalence of minor pathogen infections (especially *Corynebacterium bovis*) and use of post milking teat dip. This effect was believed to operate through increasing the prevalence of uninfected quarters which are suspected to be more prone to clinical episodes following infection (Schukken et al., 1989). Other studies have demonstrated that SCC is not a determinant of probability of an acute clinical episode (Dohoo et al., 1984).

Risk of increased culling

An Australian longitudinal study involving 11,500 cows determined that 18.6% of a dairy herd is not present the following season (Williamson et al., 1978). 9.8% of dairy herds are lost each year due to disease. Mastitis was an important cause of attrition from the herd with an average of 1.2 losses per 100 cows per year. The cure rate for subclinically infected cows decreases with increasing duration of infection (Sol et al., 1994). One study (Klastrup, 1987) found that cows with infections of more than 12 months in duration had markedly lower cure rates with dry cow therapy (DCT) than cows infected less than 12 months (37%

cured compared to more than 70%). It was not stated whether an intervening dry period or DCT had been applied in these cases. It is assumed that response to DCT after unsuccessful DCT at the end of the previous lactation is associated with very low cure rates.

A USA study investigating recurrence rates of clinical mastitis estimated the probability of occurrence of future clinical episode during the current lactation increased to 75% once two or more clinical episodes had occurred during the current lactation (Morse et al. 1987). Surveys of dairy farms have indicated that culling accounted for 45% of the total mastitis costs (Sischo et al., 1990). Studies have demonstrated that cows that experience an episode of clinical mastitis during lactation have significantly higher SCC after treatment for the clinical disease than was present beforehand, irrespective of the SCC present before the clinical episode (Dohoo et al., 1984).

Examination of the economics of replacement of clinical mastitis cows from dairy herds in the Netherlands using a Markov chain process (Houben et al., 1994) indicated that the optimal decision for most mastitic cows was to retain the cow for the following season. However, this study also demonstrated the considerable impact that episodes of clinical mastitis had upon the lactation earnings of the cow.

Risk of increased spread

The incidence of clinical episodes of mastitis due to contagious pathogens within a herd is correlated with the prevalence of infection within the herd (Hillerton et al., 1995). However, other studies have demonstrated a large variation in incidence of clinical mastitis within herds with similar prevalence of infection (Schukken et al., 1989). This difference between herds is likely to be related to variations in individual pathogen prevalence and milking management.

An Australian dry cow treatment trial found that infected cows that were quarter treated (in infected quarters only) had a new infection rate during the dry period four times that of infected cows treated in all four quarters (Mein at al., 1990). Another Australian study estimated the risk of increased spread form an infected cow to another to be 50%. This summary estimate was used as part of an economic projection and wide individual variation was recognised.

Cost of treatment

Treatment costs for a clinical case have been estimated at $121 per case in 1980 (VMRG, 1982). Using this methodology and substituting 1998 prices this cost estimate becomes $246. This is comprised of intramammary antibiotics ($15), veterinary attendance to 1 in 20 cases ($160 per visit), extra time at milking to treat and withhold of milk ($40), discarded milk ($26), reduced yield for the remainder of the lactation of 10% ($65), mortality risk of 1 in 200 cases ($850 per death), risk of culling of 7 in 100 cases ($650 per replacement), and risk of contamination of the bulk tank of 1 in 1000 cases ($1000 per occurrence). Excluding yield losses and culling losses, treatment costs alone are estimated at $93 per clinical case.

Treatment cost for subclinical cases using dry cow therapy is more difficult to estimate and is dependent upon the technique of herd treatment employed (ie blanket versus selective therapy). The total cost of dry cow therapy includes treatment, false positive and false negative cost components. Detection testing costs are assignable if individual cow cell count (ICCC) data is used to determine individual DCT requirements. The average cost of herd recording is $8.00 per cow for seven tests per year. Herd testing provides other herd management information such as individual cow milk production on the test day and the Production Index (PI), which is an age-standardised herd ranking.

Actual DCT treatment costs are estimated at $10 per cow. This is based on antibiotic cost of $2 per tube and labour requirements of $40 per hour (one operator can successfully treat 20 cows per hour).

Estimates of the costs of a false positive and false negative diagnosis were derived in Victoria during the 1980s (VMRG, 1982). A false positive was estimated to cost $12 per case, comprising treatment costs and an increased probability of infection next lactation due to insertion of intramammary products of 0.05. Using 1998 prices and similar methodology, this estimate becomes $22.30.

Cost of a false negative is estimated at $24-37 (VMRG, 1982), consisting of reduction in cure rate of 0.35 (dry cow cure rate was assumed to be 0.65 and spontaneous cure rate estimated at 0.30), increased risk of clinical episodes next lactation of between 0.2-0.5, and increased risk of spread to other cows of 0.5. It should be noted that this estimate does not include quantification of a reduction in risk of new infection in the dry period due to use of DCT. The reduction in risk for cows receiving DCT in all quarters compared to cows selectively quarter treated has been estimated to be 0.1 (Mein at al., 1990). Using the

methodology of VMRG and 1998 prices a false negative diagnosis is estimated to cost between $39 - $65. If the benefit of reduced new infections in the dry period is included the cost estimate reduces to $35 - $61.

Cost of prevention

Total expenditure on mastitis prevention is difficult to estimate due to cost partitioning problems discussed previously. Only direct prevention costs will be examined here. These costs can be classified as either prophylactic or monitoring costs.

The major prophylactic costs are teat dip, milking machine testing, and calving area maintenance costs. Teat dip use recommendations for the Australian plan to achieve low cell counts and healthy udders (Countdown Downunder) are 15 mls per cow per milking applied at every milking (Brightling et al., 1998). For an iodine teat dip the annual cost of teat dip per cow is estimated at $9.60.

The Australian Milking Machine Trades Association (AMMTA) for mastitis control (AMMTA, P.O. BOX 219, Bendigo, 3550) recommends milking machine testing biannually. An annual test is required to maintain good milk harvesting performance, thus the cost of a second machine test per year may be assigned to mastitis prevention. Current machine testing costs for a 20 unit dairy (able to milk 200 cows in under 90 minutes) are around $200 per year.

Provision of a clean calving environment typically involves shifting to a new paddock at the half way stage of calving. This cost may be estimated in terms of lost spring pasture production for one calving paddock. Each hectare used as a calving paddock for spring in Victoria would lose approximately 2 tonnes of pasture dry matter at a replacement value of $440 on an available energy basis (Hides et al., 1992). Most calving paddocks would be allocated at 200 cows to the hectare (with 10% of the herd present in the paddock at any one). Thus the extra cost of maintaining a clean calving area is estimated at $2.20 per cow.

The major monitoring cost arises from ICCC testing. This has been discussed previously.

Epidemiology of subclinical mastitis

The predominant cause of mastitis in cattle is infection of the mammary gland with microorganisms. Many risk factors have been identified and the salient features of epidemiology of mastitis in dairy cattle are discussed here.

Pathogens associated with subclinical mastitis in Australia

Many pathogens have been associated with mastitis in dairy cattle. There are two major categories of pathogens; contagious and environmental. These are summarised in Table 1 (Bramley et al., 1992).

Contagious pathogens are agents that live primarily inside udders or on teat skin such that they are associated with and originate from infected cows (Brightling et al., 1998). Contaminated milking equipment, the hands of milking personnel or suckling calves mainly spreads contagious pathogens.

Environmental pathogens are agents that originate from non mammary gland reservoirs (Smith, 1990). Mastitis due to environmental pathogens may occur due to increased exposure to the causal agent and/or a reduced host resistance to infection. DNA fingerprinting studies demonstrated limited genotypes of contagious pathogens and multiple genotypes of environmental pathogens (mainly *E. coli*) within study herds. It was concluded that most contagious pathogens originate from other infected quarters whereas most environmental pathogens arise from non cow sources (Lam et al., 1996). Some environmental pathogens, *S. uberis* in particular, may be capable of cow to cow transmission under certain circumstances (Smith, 1990; Mein et al., 1998; Brightling pers. comm.).

Table 1: Species of micro-organisms commonly associated with mastitis in dairy cattle (from Bramley et al., 1992).

Pathogen group	Micro-organism
Contagious	*Staphylococcus aureus*
	Streptococcus agalactiae
	Streptococcus dysgalactiae
	Coagulase negative staphylococci spp.
	Corynebacterium bovis
Environmental	*Streptococcus uberis*
	Pseudomonas aeruginosa
	Bacillus cereus
	Nocardia spp.
	Mycobacteria spp.
	Clostridial spp.
	Mycoplasma spp.
	Algae
	Fungi

Establishment of infection

Mastitis is inflammation of the mammary gland. The inflammatory response occurs due to glandular tissue invasion by mastitis pathogens. There are three recognised phases in the establishment of disease (Blood et al., 1989).

Invasion

Most mastitis pathogens gain entry to the mammary gland through the teat end. The likelihood of invasion is dependent upon the pathogen involved, the level of exposure of the teat end to the pathogen and the status of the teat canal.

The teat canal presents a physical and chemical barrier to invasion (Nickerson, 1987). The teat sphincter muscle produces a tight closure of the canal and the self-adhesive nature of teat canal keratin completes physical closure of the canal. The keratin layer has strong bacterial adsorptive properties and regular desquamation of this layer removes trapped pathogens from the canal. Teat canal lipids have antibacterial properties. Enzymes such as lysozyme are also found in the teat canal at significant levels (D. Williams pers. comm.). Significant neutrophil migration into the teat canal lumen has been observed (Nickerson, 1987).

Intervention studies have demonstrated the pivotal role of the teat canal in preventing invasion (Capuco et al., 1992). Removal of teat canal keratin with excessive dilation of the teat canal by a method analogous to careless insertion of an intramammary tube resulted in a five-fold increase in new infections compared to control cows.

The role of teat canal infections has recently been described (Nickerson, 1987; Schultze et al., 1978). Some pathogens are capable of existing in teal canal keratin. These can act as reservoirs of infection and have been incriminated in failure of antibiotic therapy when insufficient antibiotic is deposited in the teat canal. They also are a source of infective material if physically transported into the gland (eg following insertion of an intramammary tube or from a teat end impact).

Infection

For infection to establish, invading pathogens must be capable of adhering to the mammary epithelium, of surviving in milk and avoiding antibacterial substances and white blood cells present within milk (Blood et al., 1989). *E. coli* does not appear to adhere to the epithelium. As such, disease duration tends to be short with complete recovery following elimination of the bacteria and associated toxins (Anderson et al., 1977, Frost et al., 1984, Sterba et al., 1990). *S. agalactiae* has strong mammary epithelial adherence properties (Blood et al., 1989). *S. aureus* strains vary in their ability to adhere to mammary epithelium (Mamo et al., 1994). Adherence and incorporation into mammary epithelial cells are important virulence factors for *S. aureus* strains (Mamo et al., 1994, Almeida et al., 1996 a). Non-encapsulated *S. uberis* strains demonstrate superior ability to adhere to epithelial cells compared with encapsulated forms (Almeida et al., 1996 b). Pathogenic *S. dysgalactiae* strains have well defined adherence and mammary epithelial incorporation mechanisms (Calvinho et al., 1998).

Inflammation

In order for the invading pathogen to produce a significant disease episode, a sustained inflammatory response is necessary. This is dependent upon the invading pathogen successfully avoiding the defence mechanisms of the mammary gland. These defence mechanisms include physical removal, immune mediated destruction (humoral and cell mediated) and chemical neutralisation of pathogens.

The mechanisms used for avoiding host defences vary amongst pathogens. For example, *E coli* requires free iron for metabolic processes. Milk normally has a high content of

lactoferrin, which adsorbs free iron inhibiting proliferation of *E. coli*. Lactoferrin levels frequently are low after calving and following periods of stress. This may allow *E. coli* infections to persist long enough to produce disease episodes. The non-invasive nature of *E. coli*, dependence upon free iron, and strong chemotactic nature of endotoxin contribute to the short duration of disease observed due to this pathogen (Schultze et al., 1978, Blood et al., 1989).

Eighty-six percent of *S. aureus* strains isolated from mastitis cases in Australia possess a diffuse capsule, which may impede phagocytosis (Opdebeeck et al., 1988). *S. aureus* strains also have mechanisms for the invasion of mammary epithelial cells. This action is protective against humoral, cell mediated and chemical defence mechanisms. *S. aureus* is also capable of intracellular survival within white blood cells, resulting in release of viable bacteria upon death of the phagocyte. Macrophages may live for three months and this has been suggested as a cause for recurrence in some cases of *S. aureus* mastitis (Blood et al., 1989). A recently recognised host defence mechanism against invasion is apoptosis, or programmed death of invaded epithelial cells (Almeida et al., 1996 a). Host antibodies against alpha and beta toxins of *S. aureus* limit adherence and invasion of epithelial cells (Cifrian et al., 1996). Once established, *S. aureus* tends to produce foci of inflammation in the collecting ducts and alveoli characterised by periodic invasion of deeper tissues (Blood et al., 1989). Collection of exudate results in blockage of collecting ducts, atrophy of supplying alveoli and reversion of secretory epithelial cells to the involuted form. (Hoare, 1982).

Established *S. agalactiae* infections are characterised by intense proliferation within the lactiferous ducts. Build up of exudate and fibrosis of interalveolar tissues can produce atrophy of alveoli. Some transient tissue invasion occurs but infection is typically quickly eliminated from an individual site resulting in a series of crises within the gland (Blood et al., 1989). *S. uberis* strains possess mechanisms allowing adherence to mammary epithelial cells and extracellular proteins (eg collagen). This assists *S. uberis* invasion and stimulates an inflammatory response (Almeida et al., 1996 b).

Tissue invasion and persistence is less effective by streptococcal species compared to *S. aureus*. A strong cell mediated and humoral response is evoked by streptococcal species and bacterial numbers typically decrease as SCC increases. These observations may account for

the observed higher cure rates and lower recurrence rates for streptococcal infections compared to *S. aureus* infections (Brightling et al., 1998).

Risk factors for establishment of infection

Risk factors for the contagious pathogens are generally similar. The "five point plan" for cost effective mastitis control implemented in many dairying countries including the UK, USA and Australasia over the past few decades is based upon control of these major risk factors (Bramley et al., 1992). The risk factors for environmental pathogens are different to contagious pathogens. Increased incidence of environmental mastitis has led to development of more comprehensive mastitis control programs that address the increased risk posed by environmental pathogens. Examples of these national programs include the Seasonal Approach To Managing Mastitis (SAMM) Plan of New Zealand and Countdown Downunder of Australia. The major risk factors of modern dairy industries are discussed here.

Exposure

The risk of increased spread of mastitis from infected cows to uninfected herd mates is dependent upon the agent involved and the milking environment. Environmental pathogens such as *E. coli* and *Streptococcus uberis* are not commonly associated with cow to cow spread. A recent DNA fingerprinting study (Lam et al., 1996) indicated that spread from one cow to another is not commonly associated with environmental pathogens.

Exposure to contagious pathogens increases as mastitis prevalence increases. Various studies have demonstrated a correlation between the number of new clinical cases and the existing level of infection within a herd. Increased incidence of mastitis after calving was observed in herds that were not dry cow treated at the end of the previous lactation (Bramley et al., 1992). Greater exposure level is also the likely explanation for increased new infections in the dry period in cow's quarter treated with dry cow antibiotics compared to cows treated in all four quarters (Mein at al., 1990).

Use of effective teat disinfection practices is positively associated with low prevalence herds (Erskine et al., 1990). Earlier studies demonstrated that effective teat dipping resulted in significant reduction (50%) in the incidence of mastitis and that teat dipping was more effective in reducing mastitis incidence than sterilisation of milking clusters between cows (Neave et al., 1969). The mechanism by which teat disinfection reduces risk of new infection is not completely understood, but teat disinfection is recognised to reduce

bacterial numbers surrounding the teat orifice between milking, increase the rate of healing of teat lesions and cause improvement in teat conditions (Eden et al., 1998).

The presence of a defined culling program for mastitis has been demonstrated to be significantly associated with low BMCC herds in the USA. (Sears, 1993).

Studies have demonstrated interaction between control measures directed at reducing exposure to contagious pathogens. The use of teat dip and blanket dry cow antibiotic was significantly associated with low BMCC herds and the effect was synergistic (Erskine et al., 1990). An economic study from the UK demonstrated that herds employing teat disinfection all year round, blanket dry cow therapy and annual milking machine testing experienced least financial cost due to mastitis as indicated by loss-expenditure frontier techniques (McInerny et al., 1992).

Increased exposure to environmental pathogens follows contact of teat ends with a contaminated source. Coliforms on teat ends are transient indicating recent contamination. Sources of coliforms include bedding, calving area, udder wash water, wash cloths, chronically infected cows and the perineum of cows. *S. uberis* is a common inhabitant of the skin, lips, tonsils and belly of cows. Some cows pass large numbers of *S. uberis* in faeces. Increased teat end contact with the environment through recumbency (eg calving, milk fever etc) has been demonstrated to increase the risk of environmental mastitis (Blood et al., 1989). *S. uberis* mastitis is also positively associated with teat injuries; specifically loss of keratin from the teat canal (Lacy-Hulbert et al., 1995 b).

Teat end condition

Most pathogens gain entry to the gland through the teat canal. The level of exposure of the teat canal and degree of teat trauma are considered critical components of the pathogenesis of mastitis. Damaged teat ends promote growth of pathogens (Bramley et al., 1992) and reduce teat functionality (Mein et al., 1998). Lesions include hyperkeratinisation, cracking of the teat epithelium, excessive stripping of teat canal keratin, teat oedema, congestion, and petechial haemorrhages. Cyanosis, congestion and petechial haemorrhage formation is suspected to increase susceptibility to both contagious and environmental pathogens. *S uberis* infection is aided by reduced oxygen tension in teat ends (Mein et al., 1998).

Teat health is affected by exposure to mud and water and by milking machine factors operating at the claw (Brightling et al., 1998). The most important milking machine factors

influencing teat health are vacuum level, pulsation and liner suitability (Brightling et al., 1998; Eden et al., 1998; Mein et al., 1998).

Milking machines

Milking machines have major influence upon mastitis levels. The milking machines can impact upon the development and severity of mastitis in four ways (Bramley et al., 1992):

1. Provide a mechanism for physical transport of pathogenic bacteria between quarters and cows

2. Aid multiplication of bacteria at the teat end

3. Increase bacterial penetration of the teat end

4. Influence host defence mechanisms

Milking machines may be involved in direct spread of contagious pathogens and predispose to environmental pathogens through teat end damage. Machine factors which can have direct effects upon incidence of mastitis include vacuum levels, pulsation characteristics and liner types. Many indirect machine factors influence mastitis levels and include plumbing (position, bore, slope and angles) long milk tubes (length, and bore), inlets, air admission, reserve capacity, claws (volume and design), shell length, regulator function, reserve vacuum capacity and cluster alignment. More comprehensive discussions of milking machine functionality and impact upon mastitis are available (Mein et al., 1998, Bramley et al., 1992, and Eden et al., 1998)

Most mastitis control programs including Countdown Downunder of Australia and the SAMM plan of New Zealand recommend annual milking machine tests.

Milking technique and milking management

Milking management is strongly linked to mastitis incidence. The influence of milking management upon risk of spread is demonstrated by work in New Zealand (Woolford et al., 1983). In this study two separate sub-herds were milked in the same shed for two seasons. One sub-herd had 100% of cows infected with *S. aureus* and the other herd maintained complete freedom from infection. The infected sub herd was established through direct injection of *S. aureus* through the teat canal. This method was employed after

initial attempts to infect cows involving smearing the teats in *S. aureus* solution and normal milking routines established only 5 infections from 2184 quarter milkings.

Three periods of milking management that can influence the new infection rate are recognised (Mein et al., 1998). The pre-milking period is the interval from when cows enter the shed until the cups are applied. During this period the operator objectives are maintenance of low stress levels in the cow, good milk let down, removal of gross contamination of teats and mastitis identification. The machine milking period objectives are minimisation of teat end impacts through careful cups-on and cup removal techniques, minimal overmilking, maintenance of teat health and protection of the teat canal. The post milking period requires comprehensive teat disinfection, use of emollients when required and immediate removal of cows to areas of low risks for environmental infection such as pasture and feeding areas.

Milking mastitic and high cell count cows last is positively correlated with low BMCC herds in the USA (Hutton et al., 1991). Cup removal before the vacuum has fully dissipated from the cluster is associated with teat end impacts (reverse flow) resulting in transport of contaminated droplets of milk through the open teat canal. A poor cups on technique is also associated with excessive air admission and teat end impacts.

Undermilking has been demonstrated to increase risk of new infections and increase severity of disease in infected quarters (Mein et al., 1998). The incidence of mastitis is highest in cows that have cups on for set periods as opposed to removal of cups from cows when milk flow ceases (Natzke et al., 1978).

Teat disinfection of all teats for the duration of lactation is associated with reduced incidence of mastitis in herds. Incomplete teat coverage is a common cause of error (Mein et al., 1998; Eden et al., 1998).

Miscellaneous factors

Mastitis tendency has a slight genetic component. High production bulls appear to have higher mastitis levels in daughters; although the heritability is low (<16 %) and is correlated to milking speed and udder depth (Boettcher et al., 1998). Other studies have demonstrated variation in phagocytic efficiency varies between bulls (MacDonald et al., 1994). Use of somatic cell scores (SCS) as a selection index for mastitis has demonstrated bulls with best SCS have most favourable rates of mastitis in daughters. However, the methods used for

calculating SCS differ between countries resulting in poor correlation between results for individual bulls in different countries (Rogers et al., 1998). Incorporation of mastitis information into Australian Breeding Value (ABV) scores is expected in the near future (Brightling et al., 1998).

The effect of nutrition on mastitis is best defined for antioxidants. Supplementation of deficient animals with antioxidants such as selenium, vitamin E and vitamin A may enhance immune responses and reduce new infections. Studies have demonstrated this effect (Nagashima et al., 1995; Erskine, 1993). Other some studies failed to demonstrate a protective role of supplementation against new cases of clinical and subclinical mastitis (da Costa et al., 1997; Ndiweni et al, 1991 b;). One study demonstrated no effect of selenium supplementation upon incidence of mastitis but a positive association with reduction in mastitis prevalence (Ndiweni et al., 1991 a). Metabolic disorders such as ketosis and fatty liver syndrome may cause increases in mastitis incidence due to the inhibitory effect of ketones and free fatty acids on neutrophil function (Ndiweni et al., 1991 a).

Diagnosis of subclinical mastitis

Mastitis is inflammation of the mammary gland. In general inflammation of the mammary gland is associated with:

1. An alteration in the number of cells (bacteria, neutrophils, macrophages etc.) within milk.

2. Changes in milk composition as a consequence of altered secretory activity.

3. Changes in milk composition as a consequence of increased permeability of the blood/milk barrier.

Milk is most commonly used for the diagnosis of mastitis. A variety of milk tests are available, and all are subject to the usual problems of application of a test to a population (Burvenich et al., 1990). There is also disagreement amongst experts on the definition of mastitis (Giesecke, 1974; Giesecke et al., 1986).

Culture

The predominant cause of mastitis is infection of the mammary gland with a micro-organism; mainly bacteria. In general isolation of bacteria has been regarded as the most reliable diagnostic test, especially if a series of cultures are employed (Griffin et al., 1977; Smith et al., 1990; Burvenich et al., 1990). A recent study indicated that a single culture has high sensitivity (93%) and specificity (99%) when compared to the gold standard technique of two positive cultures from three consecutive samples for *S. aureus* infected cows (Hicks et al., 1994). However, an examination of treatment trial protocols in the UK indicated that single sample diagnostic methods were associated with increased rates of false positive and false negative diagnoses resulting in erroneous estimates of cure rates for some agents (Morant et al., 1988).

Some pathogens may reside in the teat canal only and not in the glandular portion. This population is most often transient but may occasionally enter the gland resulting in infection. The use of foremilk samples for culture may be result in isolation of teat canal residents. First foremilk samples should be excluded from culture to minimise risk of isolation of a teat canal resident (Schultze et al., 1978).

Somatic cell count

Somatic cells in milk include mammary secretory epithelial cells, squamous teat canal cells, monocytes and neutrophils. Non blood derived cells comprise around 2% of total cells (P. Brightling, pers. comm.). In the event of infection neutrophil numbers increase dramatically. It is this marked increase in neutrophils following infection that is the basis of somatic cell counting as an indirect test for mastitis (Homes et al., 1984).

Affect of infection on somatic cell count

Infection status is the most important factor affecting somatic cell counts in milk. In comparison other factors have lessor effects (Dohoo et al., 1982). The type of pathogen involved has been demonstrated to influence the magnitude of the cellular response but the degree of cellular response as a predictor of infectious agent is unreliable (Reneau, 1986).

Major pathogens such as *S. aureus*, *S. agalactiae*, other *Streptococcus spp.*, and coliforms produce higher average SCC than minor pathogen such as *C. bovis*, and coagulase-negative *staphylococci* (Reneau, 1986; Dohoo et al., 1982).

Use of SCC at the quarter level

An Australian study found that uninfected quarters had individual quarter cell counts (IQCC's) ranging from 80,000– 160,000 cells/ml and quarters infected with *S. aureus* had IQCC's ranging from 234,000-1,000,000 cells/ml (Sheldrake et al., 1983). New Zealand studies found average IQCC for quarters infected by major pathogens were: 1,811,000 cells/ml for *S. aureus*, 2,318,000 cells/ml for *S. uberis*, 3,893,000 cells/ml for *S. agalactiae* and 2,169,000 cells/ml for *S. dysgalactiae* (Holdaway et al., 1996 c).

Recent New Zealand work indicates that IQCC as a mastitis test has greater discriminative ability than sodium concentration, potassium concentration, N-acetyl-β-D-glucosaminidase (NAGase) concentration, electrical conductivity (EC), pH, lactose concentration, and α1-antitrypsin concentration. The probability of misclassifying quarters was estimated at 20% using IQCC (Holdaway et al., 1996 b) which was obtained by use of the most efficient IQCC cut point. This cut point was the individual cow cell counts (ICCC) that resulted in equivalent rates of false positive and false negative diagnoses within a herd. This was identified to be 245,000 cells/ml for major pathogens, however when individual herds were examined, the misclassification rate ranged from 13.7% to 23.9%. This difference in classification accuracy was due to differences in herd prevalence. A significant association was noted between IQCC and stage of lactation (Sheldrake et al., 1983).

The IQCC cut point that results in greatest accuracy of classification varies for individual herds and stages of lactation. The cut point increases as herd prevalence increases. The SCC cut point also increases as lactation advances (Holdaway et al., 1996 b). Use of a fixed IQCC threshold for classifying quarters is likely to be associated with increasing misclassification of quarters as lactation proceeds. (Holdaway et al., 1996 b; Natzke et al., 1972).

Use of SCC at the cow level

ICCC as a mastitis test is more accurate than tests involving sodium concentration, potassium concentration, NAGase concentration, EC, pH, lactose concentration, and α1-antitrypsin concentration in classification of cows (Holdaway et al., 1996 b). The cut point resulting in the most accurate classification of cows was 80,000 cells/ml; however this resulted in a 23% misclassification rate (Holdaway et al., 1996 b).

The number of quarters infected has a major influence upon ICCC. Cows with no infected quarters were found to have ICCC's significantly lower than cows infected in one, two or three quarters (Natzke et al., 1972). In this study cows uninfected at the time of sampling had an average ICCC of 214,000 cells/ml, cows with one quarter infected averaged 507,000 cells/ml, cows with two quarters infected averaged 701,000 cells/ml and cows with three quarters infected averaged 1,470,000 cells/ml. The ability of ICCC to correctly diagnose infection status in the cow improves as the number of quarters infected increases. One study found the ability to correctly classify cows as infected or uninfected increased from 77.9% to 92.7% as the number of infected quarters increased from one to four (Dohoo et al., 1982).

The predictive ability of ICCC in determining infection status with major pathogens in a population of cows was studied and results presented in Table 2 and Table 3 (McDermott et al., 1982). There is a decrease in sensitivity and increase in positive predictive value as ICCC thresholds are raised. There is an increase in specificity and decrease in negative predictive value as ICCC thresholds are raised.

Table 2: Probability of infection with a major pathogen within each ICCC range (from McDermott et al., 1982)

ICCC range (cells x 10^3 / ml)	Probability of infection with major pathogen
0-99	.05
100-199	.12
200-299	.33
300-399	.38
400-499	.58
500-599	.53
≥ 600	.61

Table 3: Sensitivity and specificity for determining infection with major pathogens for various ICCC thresholds (from McDermott et al., 1982)

ICCC Threshold (cells x 10^3 / ml)	Sensitivity	Specificity
100	.92	.53
200	.89	.75
300	.70	.82
400	.60	.87
500	.52	.89
600	.46	.91

Use of SCC at the herd level

The correlation between BMCC and percentage of quarters yielding mastitis pathogens has been found to range from 0.50 to 0.60 (McDermott et al., 1982). An estimate of BMCC is provided by a weighted average ICCC for the herd using individual cow milk volume as the weighting factor. This estimate, called the estimated bulk milk somatic cell count (EBMSCC) is correlated with the quarter infection rate within the herd (Holdaway et al., 1996 c). The relationship is:

Log_{10} EBMSCC = 1.888 + 0.4 * percentage of quarters infected

This equation has a correlation coefficient of 0.75. The major reason for low correlation between BMCC and quarter infection rate is BMCC is a function of both the quarter infection rate and the severity of infection. Improved correlation may occur if three or six month rolling average BMCC is used (Dohoo et al., 1982). The correlation between BMCC and herd geometric mean ICCC has been found to be higher (0.89). This may indicate that BMCC is a better predictor of the state of udder health in the herd than the prevalence of bacterial infection (Dohoo et al., 1982).

Correlation between $\log_{10}$ EBMSCC and percentage of cows infected within a herd was found to be 0.59. This is lower than the result obtained for the percentage of infected quarters (0.75) and reflects the dilution effect of milk from uninfected quarters on ICCC. The correlation between herd ICCC critical threshold and herd $\log_{10}$ EBMSCC was 0.65 (Holdaway et al., 1996 c).

Regression analysis to determine the critical ICCC threshold for herds indicated that EBMSCC, stage of lactation and average age of the herd were all significant additions to the model. In seasonal herds, the stage of lactation must be considered when examining BMCC's (Holdaway et al., 1996 c).

BMCC's have considerable variation. The coefficient of variation in BMCC taken at short intervals was found to be 30-35%. Herds with higher mastitis incidence generally have greater variation in daily and monthly BMCC (Reneau, 1986).

Causes of variation in somatic cell count

There are a number of recognised causes of variation in somatic cell counts. The most significant influence is infection status of the quarter, cow or herd. The predictive value of

an increase in SCC in the diagnosis of infection status of quarters or cows is better for major pathogens compared to environmental pathogens (Kirk et al., 1996 a).

The magnitude and duration of infection and the previous history of infection influence SCC expression (Reneau, 1986). The number of quarters infected has implications when composite samples are used to determine cow infection status. An increase in false negative diagnoses can be expected when composite cow SCC samples are used for determining infection status (Dohoo et al., 1982).

Many studies have identified an age effect upon SCC; with average SCC increasing with increasing age of the cow (Hoare, 1982; Blood et al., 1989; Sheldrake et al., 1983). More recent analysis indicates that this observed age effect is confounded. Older cows tend to have higher prevalence of infection than younger cows, due to greater lifetime exposure. The observed age affect may be due to prevalence differences between age groups (Reneau, 1986). Studies have demonstrated that the population of older cows that do not have infection do not experiences increases in average SCC with age (Natzke et al., 1972; Sheldrake et al., 1983; Dohoo et al., 1982). The variance in average SCC also increases with increasing age providing further evidence of the effect of increasing prevalence of infection with increasing age (Natzke et al., 1972). However, older cows may evoke greater cellular response following infection due to previous priming of the immune system and longer duration of infection contributing to the age related increase in SCC (Reneau, 1986).

The somatic cell counts of milk obtained from the various stages of the milking process are not equal. Somatic cell counts increase across the milking process. This increase is more pronounced in infected quarters compared to uninfected quarters (Holdaway et al., 1996 a). There is also significant diurnal variation in ICCC and BMCC within herds (Natzke et al., 1972; Dohoo et al., 1982; Dohoo et al. 1993 b; Reneau, 1986). There is variation in the amount of somatic cells produced throughout the day and from one day to the next. The coefficient of variation for ICCC taken over a short interval was found to be 30-35% and over a complete lactation the range was 69-301%. These observations have implications on the sampling technique required for accurate estimation of quarter or cow SCC. Somatic cell counts should be performed upon a proportional milk sample obtained from all stages of the milking process and aggregated from 24 hour milk production if an accurate point estimate of IQCC or ICCC is desired. At least five tests per lactation are recommended to control day to day variation and provide accuracy in classification of individual cows

(Dohoo et al., 1982). The BMCC coefficient of variation was found to be 24% on a daily basis and 4-46% on a monthly basis (Reneau, 1986).

There is a significant relationship between SCC and stage of lactation (Sheldrake et al., 1983; Dohoo et al., 1982; Holdaway et al., 1996 a; Holdaway et al., 1996 b; Holdaway et al., 1996 c). Somatic cell counts are elevated immediately after calving irrespective of infection status. This increase may last up to two weeks (Dohoo et al., 1982). Interpretation of SCC obtained from the first two weeks of lactation need to be interpreted with care. Somatic cells have been reported to increase as lactation proceeds; however this results from increased prevalence of infection as lactation proceeds. Studies examining uninfected cows over a lactation have demonstrated that there is no significant rise in SCC (Dohoo et al., 1982; Natzke et al., 1972; Sheldrake et al., 1983).

Stress is recognised as a possible cause of increase in SCC, however the magnitude of the response is small. Cows in oestrus do not experience an increase in SCC and injection of cows with corticosteroids has had mixed results on SCC (Dohoo et al., 1982). The stress observation may be mediated through poor milk let down resulting in retention of milk at the completion of milking. In subclinical and high cell count cows this is recognised as a cause of marked increase in SCC at the following milking (Mein et al., 1998; Bramley et al., 1992).

Elimination of infection

Elimination of infection from quarters decreases both the risk of spread within the herd and the risk of milk quality violations. Elimination of infection from commercial dairy herds has revolved around treatment of infected cows with culling of intractable cases. Ability to cull high SCC cows is reduced in seasonally calving herds where non pregnant status is the predominant cause of loss.

The cure rate is dependent upon the pathogen involved, the duration of infection, severity of infection, immune competence of the host and the stage of lactation. *S. aureus* is capable of tissue invasion, adhesion to epithelial cells, abscess formation, resistance to antimicrobial agents and survival in phagocytic cells resulting in high rates of chronic infection and reduced cure rates (Osteras et al., 1994; Mein at al., 1990; Blood et al., 1989). *Streptococcus spp.* do not invade glandular tissues to a significant degree and are not associated with high

rates of chronic infection (Klastrup, 1987). *E. coli* has no epithelial adhesive mechanisms and produces transient disease episodes (Anderson et al., 1977).

Increased duration of infection is associated with reduced cure rates. Infections with S. aureus of less than two weeks in duration were associated with 70% cure rates following treatment whereas infections of greater than four weeks in duration had 35% cure rates (Owens et al., 1997). Other studies have reported similar trends (Miller, 1974; Mein at al., 1990, Klastrup, 1987 etc).

Stage of lactation at time of treatment was found to be a significant factor in cure rates following infection with *S. aureus* (Miller, 1974). During lactation cure rates are lower than dry period cure rates irrespective of duration of infection for *S. aureus* (Klastrup, 1987).

Spontaneous elimination of infection

A proportion of infected cows self cure following infection. Estimates of the rate of self cure vary. Studies have indicated that the self cure rate for *S. aureus* has decreased from around 45% in the 1970's to around 25% in the late 1980's (Osteras et al., 1994). Possible explanations provided include adaptation of the mastitis bacteria to their environment and increased antibiotic resistance.

Role of therapy in elimination of infection

Antimicrobial treatment of infection can result in increased cure rates. Many treatment trials have demonstrated benefit from treatment.

Treatment at drying off

Dry cow antibiotic treatment is associated with higher cure rates than lactation therapy for many pathogens (Osteras et al., 1994). A recent treatment trial corrected for age, cow and herd effects found a cure rate of 65.8% for quarters subclinically infected with *S. aureus* (Sol et al., 1994). Cure rates in quarters decreased as SCC increased, number of other quarters infected with *S. aureus* increased and for infections in hind quarters. Cure rates for cows decreased as log SCC increased, age of cow increased and number of S. aureus infected quarters increased. Cure rates ranged from 92% for three year old cows with single quarter infections with *S. aureus* to a cure rate of 36% for eight year old cows with three quarters infected with *S. aureus* (Sol et al., 1994). Other studies found no association with quarter infection rate and cure rate following dry cow therapy (Natzke et al., 1974).

A longitudinal study of infected cows receiving dry cow therapy found that the odds ratio for dry cow treated cows to remain free of pathogens when compared to untreated controls reduced from 6.55 at calving to 3.52 at 30 days after calving (Osteras et al., 1994). The reduction in cure rate as lactation proceeds may reflect failure of dry cow therapy to evoke a complete cure or increased propensity for reinfection in cows previously infected.

Dry cow therapy has been shown to reduce dry period new infections; especially in uninfected quarters of infected cows (Natzke et al., 1974; Mein at al., 1990; Osteras et al., 1994). Dry cow therapy does not seem to reduce risk of new infections in the period immediately following calving (Berry at al., 1997).

Treatment trials involving untreated controls and placebo controls have noted an increase in minor pathogen infections after calving in the placebo treated group (Osteras et al., 1994). Treatment may have resulted in physical insertion of pathogens into the udder.

Treatment during lactation

There are contradictory findings in the literature on treatment of high SCC cows during lactation. Some studies have found treatment during lactation to be effective in reducing SCC (Storper et al., 1981; Mwakipesile et al., 1983; Ziv et al., 1985; Owens et al., 1997; Klastrup, 1987 etc). Other studies have found no significant cure following treatment (McDermott et al., 1983; Greene et al., 1991; Fox et al., 1987; Seymour et al., 1989; Timms et al., 1983 etc.). Reasons for these conflicting results probably relate to the different prevalence of pathogens, duration of infection and previous treatment history.

Streptococcus spp. demonstrated high cure rates (greater than 80%) following treatment in lactation. *S. aureus* cure rates were strongly influenced by duration of infection; with cure rates of 67-72% for infections of less than 12 months duration and a cure rate of 18% for infections of greater than 12 months duration (Klastrup, 1987). Other studies demonstrated cure rates of 70% for *S. aureus* infections of less than four weeks duration with cure rates of 35% for infections greater than four weeks in duration (Owens et al., 1997). Cure rates for *Streptococcus spp.* were 90% in this study. All isolates in this study demonstrated in vitro sensitivity to the treatment agent chosen indicating physical barriers to drug access. In vitro sensitivities are not predictors for efficacy of treatment of intramammary infections (Owens et al., 1997).

Treatment of high SCC cows in the absence of a microbiological diagnosis was found not to be economical. This was due to differences in pathogen cure rates, failure of SCC to return to low levels if pathogens were successfully eliminated and the high prevalence of culture negative high SCC cows (McDermott et al., 1983).

Cure rates demonstrated greater variation between herds than between treatment agents within herds in a multi agent treatment trial (Storper et al., 1981). Cure rates decreased in older cows. This observation was supported in other trials (Mwakipesile et al., 1983; Ziv et al., 1985). This may indicate that infections of longer duration were less responsive to treatment.

Culling

Cows that fail to respond to a single dry cow treatment and cows that experience three or more clinical cases of mastitis during a lactation are recommended for culling in national mastitis programs (Brightling et al., 1998; Eden et al., 1998). The cure rate following dry cow therapy for infections of longer than 12 months in duration is less than 18% (Klastrup, 1987). Studies have shown that cows that have more than two episodes of clinical mastitis during a lactation have a 75% chance of experiencing another episode during the same lactation (Morse et al. 1987). An Australian study found that 76% of infections at calving and nearly 70% of infections at mid lactation were in cows infected during the previous lactation (Mein at al., 1990). Culling of non responsive cases is likely to remain an important component of a herd mastitis control program.

Estimation of economic cost of mastitis for an average Victorian dairy farm

The economics of mastitis within a commercial dairy herd in Victoria in 1988 was examined using the associations previously described. A herd size of 200 cows with an average production of 220 kg per cow per year was examined for each of three peak seasonal BMCC levels. These peak BMCC levels were 200,000 cells/ml, 300,000 cells/ml and 600,000 cells/ml. The herd was assumed to be in a steady state; that is, the prevalence of mastitis infection was assumed to be constant from one year to the next.

Model assumptions

The peak BMCC in a seasonal herd tends to occur towards the end of lactation. The physical BMCC supply distribution used for each of these peak BMCC scenarios is described in Table 4

Table 4: Typical bulk milk cell count supply pattern for seasonal Victorian dairy herds with different season peak BMCC

Season peak BMCC (000's cells/ml)	200,000 cells/ml	300,000 cells/ml	600,000 cells/ml
Percentage of year above 250,000 cells/ml	0%	20%	100%
Percentage of year above 500,000 cells/ml	0%	0%	20%

The typical milk quality payment penalty scheme for bulk milk supplied to a major Victorian processing company in 1998 is given in Table 5

Table 5: Typical milk quality payment scheme for bulk milk supplied to a major milk processor in Victoria in 1998

BMCC quality band	Cents/litre
Base Price	$0.232
Premium 2 (<500,00 cells/ml)	$0.237
Premium 1 (<250,000 cells/ml)	$0.242

Other physical assumptions used in the estimation of the total economic cost of mastitis within a commercial Victorian seasonal dairy herd include:

1. Heifers comprise 25% of the herd and produce 80% of the production level of cows.

2. Infected cows produce 1.70% less litres and infected heifers produce 7.80% less litres than uninfected herd mates. Currently uninfected cows, which were infected as heifers produce 5.70% less litres than uninfected cows that were not infected as heifers (Woolford et al., 1983; Woolford, 1985).

3. The percentage of infected quarters is estimated from the correlation;

a. Percentage of infected quarters = (Log_{10}EBMCC – 1.888)/ 0.04 (Holdaway et al., 1996 c).

b. An average of 1.2 infected quarters per infected cow is assumed.

4. An average of 50% of infected cows (SCC above 250,000 cells/ml) become clinical during a single lactation (Hoare et al., 1977). The average cost of a clinical case is $93 per case. This figure excludes lost production and culling costs (VMRG, 1982).

5. Cow losses due to mastitis are set at 5% of infected cows. A culled cow incurs change over costs of $600 per case.

6. Herd testing costs average at $8.00 per cow and half of these costs are related to the generation of SCC data.

7. Milking machine testing costs $2,000 per annum with 50% of these costs assignable to mastitis.

8. Teat disinfection costs average at $9.60 per cow per year.

9. Dry cow antibiotic treatment costs of $8.00 per treated cow are applied. These costs include labour and materials. Blanket dry cow therapy is used if the herd experiences a peak BMCC for the season of 250,000 cells/ml or greater.

10. SCC's are used to diagnose infected cows with a cut point of 250,00 cells/ml. This is associated with a test sensitivity of 79% and a test specificity of 78% (McDermott et al., 1982). A false positive diagnosis incurs a cost of $14.30 per case. This cost reflects the increase in clinical episode risk of 5%. A false negative incurs a cost of $48.00 per case (VMRG, 1982).

11. The cost of running an extra calving paddock is $2.20 per cow per year.

Model physical output

The physical farm output arising from modelling these assumptions is given in Table 6

Table 6: Estimated farm physical performance for a 200 cow herd for three seasonal peak BMCC levels

Physical output (per year)	Peak farm BMCC		
	Low (200,000 cells/ml)	Medium (300,000 cells/ml)	High (600,000 cells/ml)
No. infected cows	17	37	111
No. infected Heifers	4	9	28
No. infected Cows	13	28	83
No. cows infected as heifers	4	8	23
No .clinical Cases	9	19	56
No. culls	1	2	6
No cows for dry cow therapy	54	200	200
No. false negative diagnoses	4	0	0
No. false positive diagnoses	40	163	89
Total potential production (L)	1,073,171	1,073,171	1,073,171
Total production loss (L)	3,913	9,800	16,890
Total actual production (L)	1,069,258	1,063,371	1,056,281

Model economic output

An estimation of the total cost of mastitis within this herd at the three peak BMCC levels is given in Table 7

Table 7: Estimated economic losses due to mastitis in a 200 cow herd for three seasonal peak BMCC levels

Economic losses (per year)	Peak farm BMCC		
	Low (200,000 cells/ml)	Medium (300,000 cells/ml)	High (600,000 cells/ml)
Cull cow losses	$600	$1,200	$3,600
Milk income loss	$908	$3,337	$10,256
Production loss	$908	$2,274	$3,918
Penalty payment loss	$0	$1,063	$6,338
Clinical case loss	$837	$1,767	$5,208
Dry cow therapy loss	$1,196	$3,931	$2,873
Dry cow therapy cost	$432	$1,600	$1,600
False negative cost	$192	$0	$0
False positive cost	$572	$2,331	$1,273
Shed costs	$2,920	$2,920	$2,920
Teat dip	$1,920	$1,920	$1,920
Machine test	$1,000	$1,000	$1,000
Herd testing costs	$800	$800	$800
Calving paddock costs	$440	$440	$440
TOTAL MASTITIS COST	$7,701	$14,395	$26,097
Total mastitis cost / cow	$38.51	$71.97	$130.48
Potential economic return from improved mastitis control	-	$6,694	$18,396
Potential gain / cow	-	$33.47	$91.98

The potential gains to be achieved from successful mastitis control are significant. Farms with a peak BMCC above 250,000 cells/ml are experiencing major financial losses. The implementation of a successful mastitis control program will result in sizeable returns on these farms. Conversely, further reduction in mastitis levels below a peak BMCC of 200,000 cells/ml is not likely to result in significant increase in farm profitability.

Approximately 50% of Victorian farms experience a seasonal peak BMCC above 250,000 cells/ml. Cost effective improvements in mastitis control on commercial farms is an industry priority.

Chapter 2

A clinical trial to evaluate the effectiveness of treating high somatic cell count cows during lactation

Introduction

Many Australian milk factories have introduced milk quality payment schemes that include a significant somatic cell count component. The major factor influencing BMCC is the prevalence of infected quarters in a herd (Hutton et al., 1991; Holdaway et al., 1996 c; Dohoo et al., 1982 etc.). Thus, the development of methods for treating infected quarters during lactation that produce sustained and significant reduction of ICCC has high industry priority.

Treatment of high SCC cows is more effective with dry cow antibiotic infusions at the end of lactation than treatment with antibiotics during lactation (Osteras et al., 1994; Klastrup, 1987; Miller, 1974); however, dry cow therapy does not allow immediate redress of elevated BMCC's. Studies investigating treatment of high SCC cows during lactation have presented mixed results. Some studies have indicated that treatment during lactation is effective in lowering SCC (Storper et al., 1981; Mwakipesile et al., 1983; Ziv et al., 1985; Owens et al., 1997; Klastrup, 1987). Others have indicated that treatment during lactation is not effective or economical (McDermott et al., 1983; Greene et al., 1991; Fox et al., 1987; Seymour et al., 1989; Timms et al., 1983).

Increased frequency of treatment of high SCC cows during lactation by commercial producers indicated that a formal clinical trial to evaluate the effectiveness of treating high somatic cell count cows during lactation under local conditions was required. This trial was conducted in the Macalister Irrigation District (MID) of Victoria from 1995 to 1997.

Materials and methods

A random selection of commercial herd recording farms in the Macalister Irrigation District (MID) was approached for inclusion into the study in early Spring 1995. This process continued until 50 farms agreed to participate. A total of 11,000 cows were available in the 50 selected herds. Cows were eligible for selection from within each herd if they had recently calved (Spring calving), had been submitted to commercial herd testing no less than 15 days after calving and had produced an ICCC of 500,000 cells/ml or greater from this test. This selection procedure was necessary to minimise false positive inclusions

into the study population. Somatic cell counts decrease from high levels in the first 14 days after calving in infected and uninfected cows (Dohoo et al., 1982). Studies indicate that cows with an ICCC in excess of 500,000 cells/ml 14 days after having calved have a high probability of infection with a major pathogen (McDermott et al., 1982). Using 500,000 cells/ml as a threshold this study estimated the sensitivity to be 52% and the specificity to be 89%. The likelihood ratio for a positive test was calculated as 4.73. This selection process yielded 441 eligible cows from 48 herds with two herds providing no eligible candidates. Only 438 cows were recruited. This was necessary to limit financial losses to farmers with large numbers of potentially participating cows. The farmers in this study volunteered their cows and agreed to bear the cost of discarded milk from treated cows. Where farmers were hesitant to treat all identified cows a lesser number was chosen.

Each cow was cultured by a single pooled quarter milk sample obtained by the farmer using aseptic technique. The gold standard technique for identification of mastitis pathogens is the culture of three consecutive day individual quarter milk samples with a diagnosis provided when at least two out of the three culture results are identical (Griffin et al., 1977). However, single pooled cultures were used in this study, as this is the most common and economical method used to identify mastitis pathogens in commercial situations. Following culture, cows were alternatively assigned to treatment and control groups within each herd based upon herd recording identification number. This was done to ensure that each farm had approximately equal numbers of treatment and control cows thus ensuring no individual farm suffered excessive economic losses arising from forced withhold of milk from treated cows.

Treated cows received 200 mg of cloxacillin in a long acting base (Orbenin L.C.[1]) as an intramammary application into each quarter for three treatments at 48-hour intervals. Treated cows also received 200 mg of erythromycin as an injection (Gallimycin-200[2]) for three treatments at 24-hour intervals, with the first injection coinciding with insertion of the first intramammary tube. Control cows received no treatment.

All remaining cows were recultured by a single pooled milk sample collected by the farmer around six weeks after treatment. This time interval was chosen to ensure that all remaining antibiotic was eliminated from the treatment cows and to accommodate the seasonality of

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[1] Orbenin L.C. Pfizer Animal Health, 38-42 Wharf Road, West Ryde, NSW, 2114

[2] Gallimycin-200. Merial Australia Pty Limited, 54-68 Ferndell Street, South Granville, NSW 2142

the farmer workload (ie after turning the bulls into the herd). All cows were monitored for the remainder of the 1995/96 lactation and for the first half of the 1996/97 lactation through commercial herd recording. All cows were exposed to usual culling procedures during the course of the trial.

Statistical analysis

Statistical analyses were conducted using Microsoft® Excel 97 (Microsoft Corporation, Redmond, Washington), SAS for Windows version 6.12 (SAS Institute, Cary, North Carolina, USA), EpiInfo 6.04b (Centers for Disease Control and Prevention, Atlanta, Georgia), PEPI 3.0 (USD Inc., Stone Mountain, Georgia), and Statistica™ for Windows Version 5.1 (StatSoft Inc., Tulsa, Oklahoma).

Results

Farm level data

The trial farm population is summarised in Table 8.

Table 8: Trial farm population

Total no. animals	No. farms	No. trial cows	Avg. herd size	Max. herd size	Min. herd size	Avg. no. trial cows per farm	Max. no. trial cows per farm	Min. no. trial cows per farm
11000	50	428	206	724	52	8.9	30	0

The fate of the 50 selected farms over the duration of the trial is summarised in Table 9. Thirty-five herds provided complete culture and herd test data for the duration of the trial with 41 herds providing complete herd test data. Six herds failed to collect milk samples with the majority of failures occurring at the second sample.

Table 9: Fate of trial farms over the duration of the study

No. of trial farms selected	No. herds with complete culture and BMCC data	No. herds with no eligible cows	No. herds dispersed	No. herds moved	No. herds with change in share farmer	No. herds ceased herd testing	No. herds with incomplete sample collection
50	35	2	2	1	2	2	6

Cow level data

A total of 312 trial cows provided milk samples for culture on both occasions. The majority of cows with incomplete samples were missing the second sample. Two hundred and thirty one trial cows provided both samples without contamination. A total of 386 trial cows contributed sufficient ICCC records for analysis. The distribution of pathogens at each culture and the average ICCC at recruitment into the trial is given in Table 10 and Figure 1. There was little variation in average ICCC between the different pathogen groups.

Table 10: Distribution of pathogens at first and second culture and average ICCC prior to the first culture period

Culture result	Number at First culture	Number at Second culture	Average ICCC prior to first culture (000's cells/ml)
Failed to sample	70	80	1,843
S. aureus	117	91	1,478
S. uberis	36	12	1,397
S. dysgalactiae	13	11	1,682
Coag. neg. Staph	9	16	1,718
Other	13	5	1,388
No growth	119	147	1,438
Contaminated	51	50	1,570
Culled	-	16	-

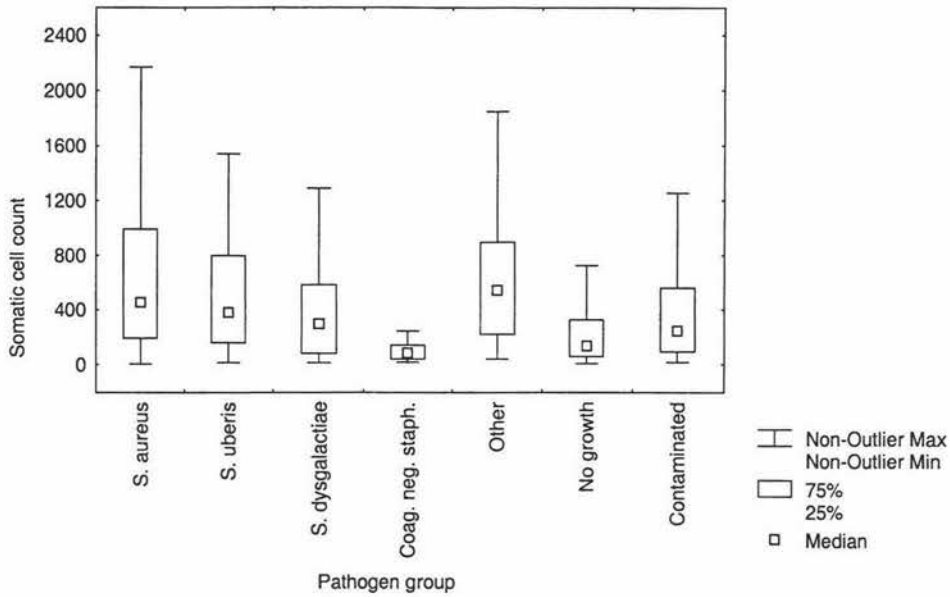

Figure 1: Box plots of somatic cell count distribution for herd tests occurring before treatment for each pathogen group (at initial culture)

The change in culture status for treatment and control groups is given in Table 7. There was no significant treatment effect ($\alpha = 0.05$) for all pathogens combined (ie pathogen at first culture, no growth at second culture); ($\chi^2 = 0.66$, 1 df, $p = 0.72$). There was also no treatment effect for individual pathogens ($\alpha = 0.05$), although the small number of cases for individual pathogens resulted in low power.

Table 11: Culture status at first and second sampling periods for treatment and control groups

Culture status at first sampling	Treatment group (t = treatment, c = control)	No Growth	No change in culture status	Other pathogen(s)
S. aureus	t	11	30	8
	c	16	29	4
S. uberis	t	8		3
	c	4	3	6
S. dysgalactiae	t	2	1	1
	c	2	1	1
Coag. neg. staph.	t	2	1	1
	c		1	
Other	t	5		1
	c	1		1
No growth	t	35	-	15
	c	33	-	5
Contaminated	t	11	6	
	c	7	7	3
Pooled	T	28	32	14
	c	23	34	12

Examination of cow ICCC data for the lactation preceding treatment (1994/95 lactation) was undertaken in order to classify infection status at the time of treatment. A trialist was given an infection status of 'chronic infection' in the 1995/96 lactation if she experienced one or more ICCC's equal to or above 250,000 cells/ml in the 1994/95 lactation. A trialist was given a status of 'new infection' in the 1995/96 lactation if all of her ICCC's in the 1994/95 lactation were below 250,000 cells/ml or if the 1995/96 lactation was her first lactation. A total of 380 trial cows could be classified according to previous lactation infection status. Of these, 285 provided both culture samples and 213 provided both culture results without contamination; of these 75 cows were new infections and 138 cows were chronic infections. The distribution of pathogen types for newly infected and chronically infected subgroups at initial culture is given in Table 12. *Staphylococcus aureus* was more prevalent in the chronically infected group and there was a higher prevalence of 'no growth' 'in the newly infected group.

Table 12: Number and prevalence of individual pathogen types (as isolated at initial culture) for newly infected and chronically infected trialists.

Pathogen isolated at culture 1	New Infections		Chronic Infections	
	No. of cows	Prevalence	No. of cows	Prevalence
S. aureus	24	23.1%	85	48.0%
S. uberis	10	9.6%	22	12.4%
S. dysgalactiae	4	3.8%	7	4.0%
Coag. neg. staph.	6	5.8%	2	1.1%
Other	5	4.8%	5	2.8%
No growth	55	52.9%	56	31.6%
Total	104		177	

The effect of treatment upon bacteriological cure for new infections in the 1995/96 lactation is given in Table 13. The result for chronic infections is given in Table 14. There was no significant treatment effect ($\alpha = 0.05$) for any individual pathogen or for all pathogens combined in either the newly infected or chronically infected groups.

Table 13: Culture status at first and second sampling periods for treatment and control groups for cows classified with new infections during the lactation of treatment (1995/96)

Culture status at first sampling	Treatment group (t = treatment, c = control)	No Growth	No change in culture status	Other pathogen(s)
S. aureus	t	3	4	3
	c	6	5	3
S. uberis	t	4		2
	c	1	1	2
S. dysgalactiae	t	2		1
	c		1	
Coag. neg. staph.	t	1		3
	c		1	1
Other	t	1		2
	c	1		1
No growth	t	20	-	9
	c	15	-	11
Contaminated	t	4	2	1
	c	5	3	4
Pooled	t	35	6	21
	c	28	8	22

Table 14: Culture status at first and second sampling periods for treatment and control groups for cows classified with chronic infections during the lactation of treatment (1995/96)

Culture status at first sampling	Treatment group (t = treatment, c = control)	No Growth	No change in culture status	Other pathogen(s)
S. aureus	t	8	24	13
	c	8	20	12
S. uberis	t	4		4
	c	3	2	9
S. dysgalactiae	t		1	2
	c			4
Coag. neg. staph.	t	1	1	
	c			
Other	t	3		2
	c			
No growth	t	20	-	9
	c	15	-	12
Contaminated	t	7	3	6
	c	2	4	6
Pooled	t	43	26	36
	c	28	22	43

Four farms experienced multiple quarter mastitis outbreaks in the treated cows following the course of intramammary treatment. A total of 28 out of 214 treated cows experienced a clinical episode of mastitis in the period immediately following treatment (ie an incidence of 13.1%; 95% CI 8.9% - 18.4%). Only 5 out of 214 control cows developed mastitis over the same time period (an incidence of 2.3%; 95% CI 0.8% - 5.4%). This difference in incidence of clinical mastitis between the two groups was significant ($\chi^2 = 17.37$, 1 df, $p < 0.001$). These infections were most likely caused by the introduction of infection into the udder via poor intramammary treatment technique. Most of these infected cows did not respond to further treatment.

Examination of the trialists that provided no growth at the first culture revealed that 15 out of 50 treated cows had a pathogen isolated at the second culture, whereas 5 out of 38 control cows isolated a pathogen at the second culture period. This difference was also significant at $\alpha = 0.1$ ($\chi^2 = 3.487$, 1 df, $p = 0.06$).

The effect of treatment upon ICCC for the remainder of the lactation of treatment for each pathogen type is summarised in Table 15 and Figure 2. There appears to be little difference between treated and control groups for each pathogen type except *S. dysgalactiae*.

Table 15: Average ICCC and percentage of total ICCC's less than 250,000 cells/ml for each pathogen and treatment group over the remainder of the lactation of treatment

First culture	Average ICCC		Percentage of ICCC's less than 250,000 cells/ml	
	Control	Treatment	Control	Treatment
S. aureus	885	587	23.2%	44.8%
S. uberis	642	580	29.2%	36.5%
S. dysgalactiae	800	246	8.0%	77.4%
Coag. neg. Staph.	634	109	83.3%	96.6%
Other	464	767	44.4%	21.7%
No growth	357	278	67.2%	70.7%
Contaminated	430	489	53.8%	49.1%

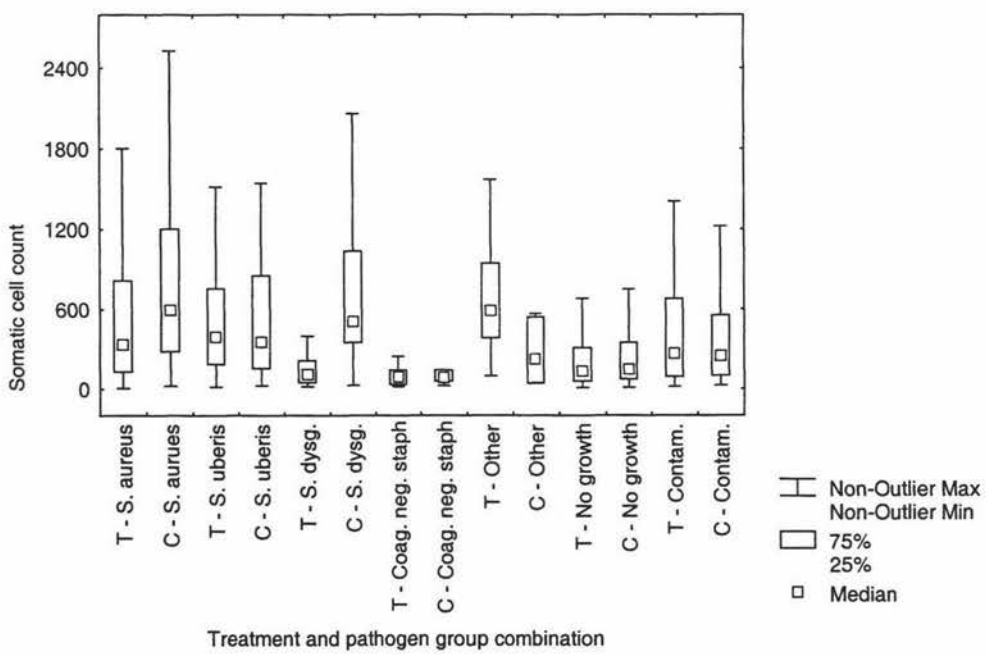

Figure 2: Box plots of somatic cell count distribution for herd tests occurring after treatment but within the lactation of treatment for each pathogen group (at initial culture) and treatment combination

The effect of treatment upon ICCC in the subsequent lactation for each pathogen type is summarised in Table 16 and Figure 3. There is no apparent difference between the treatment and control groups for each pathogen type.

Table 16: Average ICCC and percentage of ICCC's less than 250,000 cells/ml for each pathogen and treatment group for the lactation following treatment

First culture	Average ICCC (000's cells/ml)		Percentage of ICCC's less than 250,000 cells/ml	
	Control	Treatment	Control	Treatment
S. aureus	713	573	39.5%	40.6%
S. uberis	684	331	47.3%	54.1%
S. dysgalactiae	492	323	39.1%	51.7%
Coag. neg. Staph.	117	138	100.0%	93.3%
Other	326	608	54.5%	39.1%
No growth	282	331	77.8%	62.7%
Contaminated	437	685	53.2%	53.2%

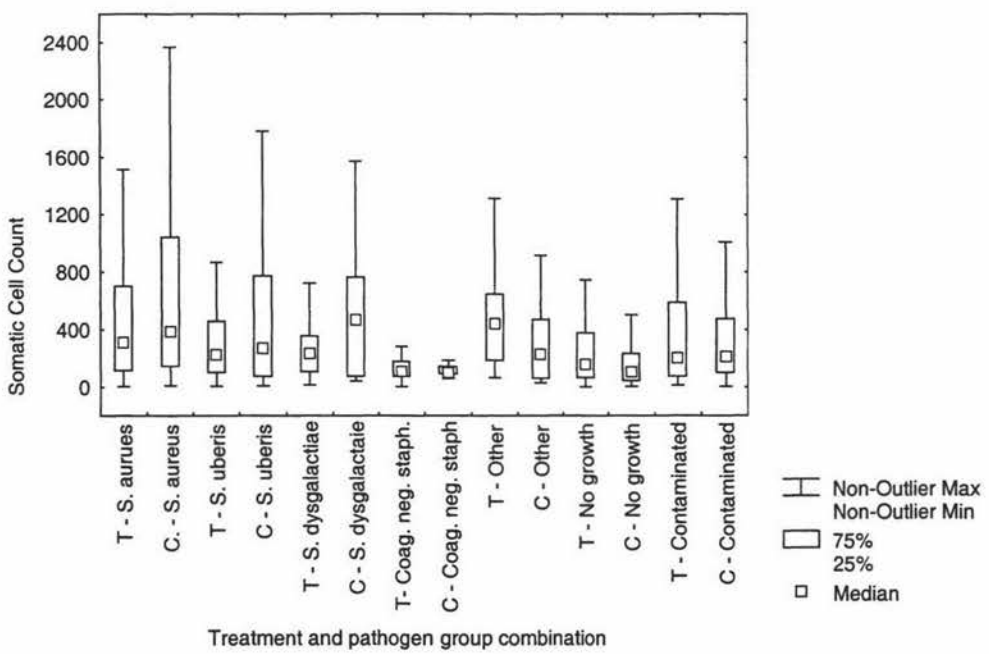

Figure 3: Box plots of somatic cell count distribution for herd tests occurring in the lactation following treatment for each pathogen group (at initial culture) and treatment combination

The effect of treatment upon ICCC for the remainder of lactation for newly infected and for chronically infected cows was examined. Results are summarised in Table 17 and Figure

4. There was no apparent difference due to treatment in either the newly infected group or the chronically infected group. The newly infected cows had a lower ICCC than chronically infected cows after treatment, although both treated and untreated groups displayed significant lowering of ICCC's.

Table 17 : Average ICCC and percentage of ICCC's less than 250,000 cells/ml for newly infected and chronically infected cows within each treatment group for the lactation of treatment

Infection status	Average ICCC (000's cells/ml)		Percentage of ICCC's less than 250,000 cells/ml	
	Control	Treatment	Control	Treatment
New infections	318	215	67.6%	80.0%
Chronic infections	860	633	25.1%	38.9%

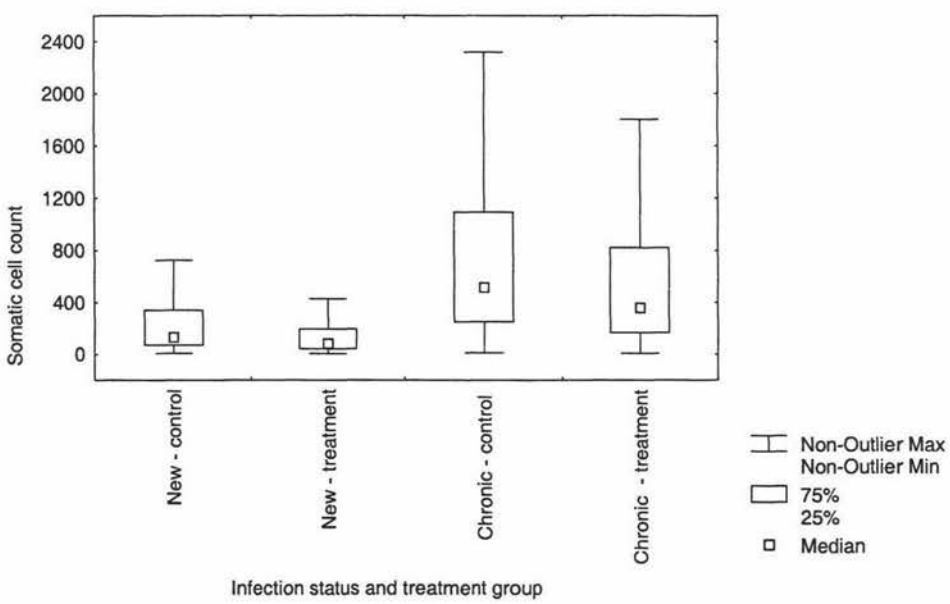

Figure 4 : Box plots of somatic cell count distribution for herd tests occurring in the lactation of treatment for each infection status and treatment combination

The effect of treatment upon ICCC in the lactation following treatment for newly infected and for chronically infected cows was examined. Results are summarised in Table 18 and Figure 5. There was no apparent difference due to treatment in either the newly infected group or the chronically infected group. Once again, the newly infected group had an

average ICCC much lower than that of the chronic group, but this difference could not be attributed to treatment.

Table 18 : Average ICCC and percentage of ICCC's less than 250,000 cells/ml for newly infected and chronically infected cows within each treatment group for the lactation following treatment

Infection status	Average ICCC (000's cells/ml)		Percentage of ICCC's less than 250,000 cells/ml	
	Control	Treatment	Control	Treatment
New infections	245	268	78.0%	72.6%
Chronic infections	732	634	35.7%	39.7%

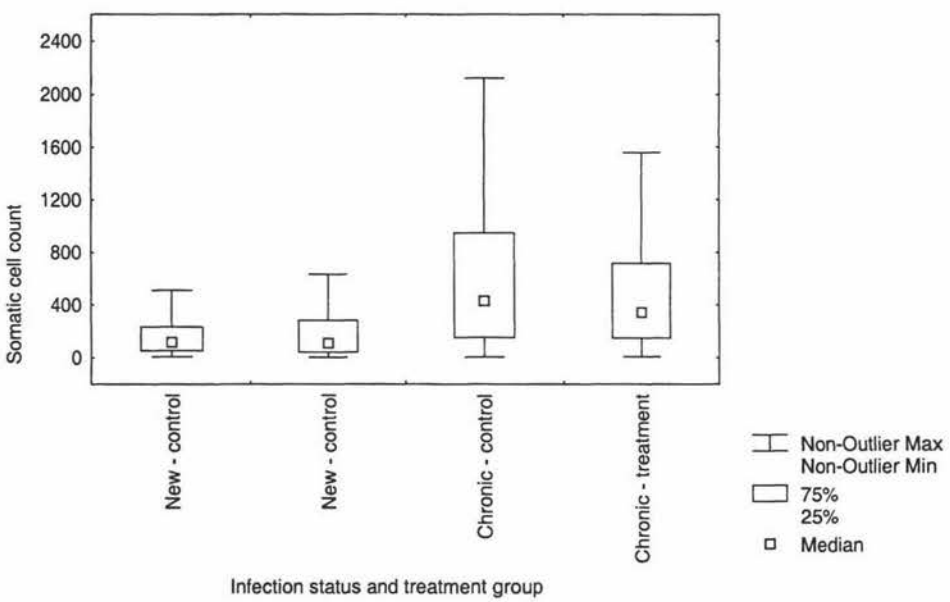

Figure 5 : Box plots of somatic cell count distribution for herd tests occurring in the lactation following treatment for each infection status and treatment combination

Trial cows were more likely to be culled from the herd than non-trialists. The fates of the 8,900 trialists were compared in Table 19. The fates of the 8,900 non-trialist Spring 1995 calving herd mates from the 35 eligible cell counting herds were also compared to the trialists in Table 19. Trial cows were 2.12 times as likely to be removed from the herd as non-trialists. It should be noted that older cows would be over-represented in high somatic cell count cows. Older cows are also at greater risk of being culled than younger cows, thus the univariate risk ratio will also over-estimate the culling pressures arising solely due to

mastitis. Use of Mantel-Haenszel techniques to control for the confounding effect of age resulted in a summary odds ratio for culling of trialists compared to unaffected Spring calving herd mates of 1.87 (95% CI 1.47 – 2.38). Treated cows had an identical survival pattern to control cows.

Table 19: Survival of treatment, control and eligible non-trialist cows from initial treatment time to begin the lactation following treatment

Group	Lost from herd	Survived to begin next lactation	Relative risk	Relative risk ratio and 95% CI for survival compared to treatment group	$\chi 2$ comparison to treatment group
Treatment	58	135	0.30	-	-
Control	58	135	0.30	1 (0.74 – 1.36)	0 (p=1)
Non trialists	1263	7640	0.14	2.12 (1.70 – 2.64) *	38.30 (p<0.001)

Treatment had no significant ($\alpha = 0.05$) effect on survival to the next lactation for each of the pathogen groups; however it should be noted that there were few cases in each pathogen group resulting in low power. This is summarised in Table 20.

Table 20: Survival, odds and odds ratio of survival of treatment and control cows for each pathogen (as isolated at the first culture)

First culture result	Treat. group	No. survive in herd	No. lost from herd	Relative risk of survival	Risk ratio and 95% CI for survival	χ^2 comparison of treatment and control groups
S. aureus	c	35	25	0.58		
	t	32	35	0.56	0.96 (0.70-1.32)	0.06 (p=0.81)
S. uberis	c	12	7	0.63		
	t	9	8	0.53	0.84 (0.48-1.47)	0.39 (p=0.53)
S. dysgalactiae	c	3	3	0.50		
	t	4	3	0.57	1.14 (0.41-3.19)	0.07 (p=1)
Coag. neg. Staph.	c	1	2	0.33		
	t	3	3	0.50	1.50 (0.25-8.98)	0.22 (p=1)
Other	c	3	1	0.75		
	t	4	5	0.44	0.59 (0.24-1.49)	1.04 (p=0.56)
No growth	c	32	24	0.57		
	t	46	17	0.73	1.28 (0.97-1.68)	3.31 (p=0.07)
Contaminated	c	18	9	0.67		
	t	15	9	0.63	0.94 (0.41-3.19)	0.10 (p=1)

The effect of infection status on survival is given in Table 21. Newly infected cows were more likely to survive in the herd than chronically infected cows; although this difference was not significant. Treatment had a significant effect upon the survival rate for newly infected cows (χ^2 = 4.58, p = 0.03). The magnitude of this effect was to approximately halve the loss rate (42% reduced to 25%). Treatment did not have a significant effect on survival of chronically infected cows.

Table 21 : Survival to the lactation following treatment for newly infected and chronically infected cows and for infection status-treatment subgroups

Group	Lost from herd	Survive in herd	Relative risk of loss	Risk ratio	95% CI of risk ratio	χ^2 statistic	P value
New	50	99	0.34	0.8	0.61-1.05	2.72	0.1
Chronic	97	134	0.42				
New treated	18	54	0.25	0.6	0.37-0.97	4.58	0.03
New untreated	32	45	0.42				
Chronic treated	49	70	0.41	0.96	0.71-1.30	0.07	0.8
Chronic untreated	48	64	0.43				

Multivariate analysis

Repeated measures analysis of variance was used to examine the effects of treatment upon SCC for the remainder of the lactation of treatment and for the subsequent lactation. SAS PROC MIXED was used for analysis. Log10 somatic cell count was the dependent variable. Transformation was performed to aid parametric analysis (Ali et al., 1980).

Fixed effects included in the model were treatment, herd test number (up to a maximum of six herd tests), previous lactation infection status, lactation year and days in milk. Lactation year and days in milk were included as covariates. Herd was included as a random effect. Individual cow (cow ID) nested within lactation number and the interaction term herd by treatment were also included as random effects. Individual herd tests for each cow within each lactation provided the repeated effect measure. A compound symmetry covariance structure was used to model the within-animal variation and results are given in Table 22.

The herd and the repeated measures effect of cow (Cow ID) within lactation year (LactYear) were significant random effects. There was no apparent interaction between herd and treatment indicating that the treatment effect did not vary between farms.

Table 22: Covariance parameter estimates

Covariance parameter	Subject	Estimate	Standard error	Z value	Prob (z)
Herd		0.03460	0.01317	2.63	0.0043
Herd * Treatment		0.009804	0.006214	1.58	0.0573
CS	Cow ID (LactYear)	0.1117	0.009037	12.36	<0.0001
Residual		0.1511	0.004284	35.27	<0.0001

Results of examination of fixed effects are given in Table 23. Significant fixed effects were herd test number (TestNo), lactation year (LactYear), number of days in milk (LactDays), previous lactation infection status (PrevLact) and the interaction term lactation year by herd test number (LactYear * TestNo). Treatment did not have an overall effect, but the interaction term treatment by lactation year (Treatment * LactYear) was significant. There was no significant three-way interaction term in the final model.

Table 23: Tests of fixed effects

Effect	Numerator DOF	Denominator DOF	F value	Prob > F
TestNo	5	2495	95.33	<0.0001
Treatment	1	30.3	1.00	0.3253
TestNo * Treatment	5	2512	2.94	0.0119
LactYear	1	505	37.98	<0.0001
LactYear * Treatment	1	838	6.88	<0.0089
LactYear * TestNo	5	2529	81.59	<0.0001
LactDays	1	2563	213.01	<0.0001
PrevLact	1	591	166.13	<0.0001

The actual effect of group membership is examined in Table 24. Three-way interaction terms are not included, as they were not found to be significant in the final model. Analysis group means have been converted back from the log10 scale to provide mean SCC estimates (after controlling for the model random effects and the effects of other variables).

Table 24: Least square means for fixed effects for different treatment, herd test number and lactation year combinations

Effect	Lactation year	Test No.	Treatment	PrevLact	Estimate (log10 SCC)	SE	SCC Estimate
Treatment			c		2.3883	0.0432	245
Treatment			t		2.3481	0.0425	223
TestNo		1			2.7341	0.0412	542

TestNo		2			2.4352	0.0408	272
TestNo		3			2.3347	0.0407	216
TestNo		4			2.2661	0.0410	185
TestNo		5			2.2371	0.0416	173
TestNo		6			2.2017	0.0438	159
TestNo * Treatment		1	c		2.7082	0.0486	511
TestNo * Treatment		1	t		2.7601	0.0474	576
TestNo * Treatment		2	c		2.4288	0.0482	268
TestNo * Treatment		2	t		2.4416	0.0473	276
TestNo * Treatment		3	c		2.3751	0.0483	237
TestNo * Treatment		3	t		2.2943	0.0472	197
TestNo * Treatment		4	c		2.3002	0.0486	200
TestNo * Treatment		4	t		2.2319	0.0476	171
TestNo * Treatment		5	c		2.2784	0.0493	190
TestNo * Treatment		5	t		2.1959	0.0485	157
TestNo * Treatment		6	c		2.2389	0.0528	173
TestNo * Treatment		6	t		2.1645	0.0520	146
LactYear	95		c		2.4724	0.0397	297
LactYear	96		t		2.2639	0.0432	184
LactYear * Treatment	95		c		2.5312	0.0461	340
LactYear * Treatment	95		t		2.4136	0.0453	259
LactYear * Treatment	96		c		2.2453	0.0516	176
LactYear * Treatment	96		t		2.2826	0.0502	192
Treatment			c		2.3883	0.0432	245
Treatment			t		2.3481	0.0425	223
LactYear	95				2.4724	0.0397	297
LactYear	96				2.2639	0.0432	184
LactYear * Treatment	95		c		2.5312	0.0461	340
LactYear * Treatment	95		t		2.4136	0.0453	259
LactYear * Treatment	96		c		2.2453	0.0516	176
LactYear * Treatment	96		t		2.2826	0.0502	192
PrevLact				0	2.1621	0.0420	145
PrevLact				1	2.5743	0.0402	375

Whilst treatment did not have an overall effect, the interaction between treatment and lactation year was significant. Treatment produced a lowering of SCC for the lactation of treatment (1995). However, the magnitude of this SCC reduction was not substantial with the average SCC of treated cows remaining above 300,000 cells/ml. There was no significant effect of treatment on SCC for the lactation following treatment (1996). The statistical tests of lactation year and treatment group combinations are given in Table 25.

Table 25: Effect of treatment group and lactation year slices

Effect	Lact. Year	DOF	DOF	F Value	Prob > F
LactYear*Treatment	95	1	50	6.73	0.0124
LactYear*Treatment	96	1	85.2	0.48	0.4921

Discussion

Treatment of high SCC cows during lactation offers little commercial advantage to dairy farmers. This finding supports previous conclusions from similar work conducted in other countries (McDermott et al., 1983; Greene et al., 1991; Fox et al., 1987; Seymour et al., 1989; Timms et al., 1983).

Bacteriological cure

There was no significant treatment effect upon bacteriological cure rates when all pathogens were grouped. No treatment effect was observed when cases were analysed according to previous lactation infection status (ie. new infections versus chronic infections). This result precludes the use of ICCC data alone to identify potentially infected cows for treatment during lactation.

Ninety-eight cases of *S. aureus* were available for analysis and no treatment effect was observed. There was no effect of treatment in either newly infected or chronically infected *S. aureus* subgroups. Treatment during lactation of *S. aureus* infected cows is not effective at producing bacteriological cure.

Low power was present when most other individual pathogens were analysed. This may have prevented the effect of treatment on streptococcal infections to be adequately demonstrated. The treatment of streptococcal infections during lactation is recognised to be effective at eliminating infection as demonstrated by the use of blitz therapy for *S. agalactiae* infected herds (Brightling et al., 1998). In absolute terms, 10 out of 15 treated streptococcal infections were cured whereas 6 out of 17 untreated streptococcal infections were cured following treatment (44% power). There was a high spontaneous cure rate in untreated *S. uberis* infected cows (this also contributed to the absence of an observed treatment effect). *S. uberis* is an opportunistic invader of the mammary gland, as such, it may not possess effective persistence mechanisms resulting in high rates of self-cure. Persistence in *S. uberis* strains is related to the presence or absence of a capsule; with non-encapsulated forms demonstrating more effective epithelial adhesion (Almeida et al., 1996 b). The ability of cows to self-cure when infected with S. uberis should not be underestimated when examining and interpreting culture data from problem herds. The high prevalence of no growth observed in the newly infected group of cows may have been due to high rates of self-cure in *S. uberis* infected cows.

Use of a single pooled sample for culture has acceptable diagnostic accuracy for the identification of infection status of quarters if sample collection is performed to high standard. The gold standard technique for identification of mastitis pathogens is the culture of three consecutive day individual quarter milk samples with a diagnosis provided when at least two out of the three culture results are identical (Griffin et al., 1977). Studies have demonstrated that a single sample can approach diagnostic accuracy of the gold standard (Hicks et al., 1994). This study found *S. aureus* infected quarters could be diagnosed with the following test parameters; 93% sensitivity, 99% specificity, likelihood ratio of a positive test of 2.9, and the likelihood ratio of a negative test of 0.03. This estimation is supported by examination of the data from Griffin et al., 1977. The single-sample diagnostic test parameters for each pathogen from this study are given in Table 26. Sensitivity was above 90% and specificity was above 98% for all major pathogens examined.

The use of a single sample at both collection times was associated with a high loss rate of culture data for analysis. A total of 312 cows provided both milk samples for culture, but 81 of these cows were precluded from analysis due to the presence of contamination at one or both samples. The average contamination rate over the study was 14.6% (14.2% for the first collection period and 15.1% at the second collection period). A total of 27.8% of cows would be excluded from analysis through the provision of contaminated samples at one or both collection periods (or, 87 cows from 312) assuming an average contamination rate of 14.6% with independence between culture events. If the three samples per period methodology had been used, the loss rate due to contamination would be expected to reduce to 4.2% (or, 13 cows from 312). This represents a reduction in risk of loss of around 85%. The use of a single sample technique reduced the power of the study.

Table 26: Estimated single sample culture diagnostic test parameters as estimated from Griffin et al., 1977.

Pathogen	Sensitivity	Specificity	Prevalence in study	Positive Predictive value (PPV)	Negative Predictive value (NPV)
S. aureus	90.1%	98.1%	16.0%	90.0%	98.1%
S. agalactiae	98.1%	99.9%	3.8%	97.5%	99.9%
S. dysgalactiae	91.9%	99.5%	2.5%	80.6%	99.5%
S. uberis	96.2%	99.6%	3.5%	89.1%	99.6%

A pooled sample was used to represent infection status of each cow. This is likely to be associated with a loss of overall sensitivity. Problems may arise due to; low volumes of quarter milk samples taken, an increase in the frequency of contamination of samples following prolongation of collection times and classification difficulties arising from multiple quarter infections in individual cows with more than one pathogen. Poor collection technique will also compromise result quality. In this study, the farmer following detailed instruction collected the pooled milk samples.

Somatic cell count

Whilst there was a statistically significant SCC lowering effect of treatment in the multivariate model, the magnitude of this reduction was not great. The average SCC of untreated cows for the remainder of the lactation was 377,000 cells/ml whereas, treated cows had an average SCC of 303,000 cells/ml over the remainder of the lactation of treatment (controlled for random effects and other fixed effects in the model). The treatment effect also did not persist beyond the lactation of treatment. In the subsequent lactation the average SCC of surviving treated cows was 211,000 cells/ml, whereas the average SCC of surviving control cows was 191,000 cells/ml.

Only *S. dysgalactiae* infected cows demonstrated satisfactory ICCC reduction following treatment. This effect was not significant, but low power was again present. The effect of treatment was lost by the following lactation. There was no treatment effect upon ICCC in *S. uberis* infected cows, although number of cases was again low. Insufficient *S. agalactiae* infected cows precluded analysis. Both treated and untreated *S. uberis* infected cows persisted with high ICCC's for the remainder of the lactation of treatment but ICCC's returned to acceptable levels in both groups in the subsequent lactation. Treatment during lactation offered no advantage over the use of dry cow therapy at the end of lactation.

Survival

There was no effect of treatment upon simple survival rates of cows to the subsequent lactation with both treated and untreated cows demonstrating identical risk of loss from the herd. Unaffected Spring calving cows were 2.12 times more likely to survive to the subsequent lactation than either treated or control cows (95% CI 1.70 - 2.64). If both treatment and control cows are pooled this confidence interval reduces to 1.80 - 2.49. When the confounding effects of age are controlled for by use of Mantel-Haenszel techniques, the summary odds ratio was 1.87 (95% CI 1.47 – 2.38). Survival rates did

appear to improve following treatment in newly infected cows; the relative risk of culling in the treated cows compared to untreated cows was 0.60 (95% CI 0.37-0.97).

A significant number of cases of multi quarter clinical mastitis occurred in treated cows. Twenty-eight cases were reported from 214 treated cows, whereas only 5 cases were identified from 214 control cows. This represents an incidence of new cases following treatment of 13.1% (95% CI 8.9% - 18.4%). It is most likely that the use of intra-mammary tubes introduced new infection to these cows. The farmers performed all treatments; and treatment occurred during the course of a normal milking. Most cases of clinical mastitis occurring after tube insertion failed to respond to ongoing treatment.

There is a need to increase farmer awareness of the requirements for sterility when inserting intra-mammary tubes into the udders of cows. This includes aspects of teat preparation, tube insertion, restraint of the patient and teat disinfection after treatment. All of the relevant farmers in this study rapidly identified the outbreak of clinical mastitis in treated subclinical cows in their herds. However, the effect of improper tube insertion in individual cows with clinical mastitis would be much less obvious to the farmer – these cases would appear to the farmer to not respond to initial antibiotic therapy. It is most likely that a significant number of cows with clinical mastitis have new pathogens introduced into the udder with the initial course of intra-mammary antibiotic tubes. These introduced pathogens can result in a prolongation of the course of clinical disease, a prolongation of treatment and a reduction in overall cure rates. Poor intra-mammary tube insertion technique with dry cow antibiotic can also result in an increase in the incidence of clinical disease following treatment. Unless recently dried off cows are closely observed in the days immediately following treatment, new cases of clinical mastitis will not be discovered.

Chapter 3

Development of baseline data necessary for the production of a stochastic computer simulation model of mastitis in Australian dairy herds

Introduction

The Australian national mastitis control program, Countdown Downunder (CDDU), was launched in 1999 with the primary objective of lowering the average national SCC. The cost effective delivery of required information to farmers and service providers on a regional basis is essential for success. In order to objectively evaluate the impact of the program and to identify the components of the program that require greatest emphasis on a regional basis, the Dairy Research and Development Corporation (DRDC) commissioned the development of an Australian mastitis computer simulation model.

The computer simulation model of mastitis in Australian dairy herds will be required to:

1. Allow accurate estimation of the likely cost-benefits from implementation of CDDU control program on a herd, regional and national level.

2. Identify the critical control points for the management of disease on a regional basis. This will allow the CDDU program to be tailored to meet regional requirements.

3. Identify areas for future research

4. Determine the likely milk quality outcomes from implementation of the CDDU program in regional Australia.

The development of a simulation model comprises three major phases:

1. The derivation of the structure of the model along with underlying equations, transition probabilities and decision algorithms.

2. Computer programming to produce a stand-alone application

3. Verifying, validating, testing and refining the final model.

The author was contracted to assist derive the structure of the model, the underlying equations, transition probabilities and decision algorithms. The development of a sub model that predicts milk yield and cell count within cows is the major subject of chapter

three. The presentation of the final mastitis model structure and a summary of the available transition probabilities and decision algorithms is provided to assist further model development. Completion of the model development is to follow with the involvement of computer programmers.

Materials and methods

The two current major pathogens of the Australian dairy industry are *Staphylococcus aureus* and *Streptococcus uberis*. Each of these pathogens was modeled.

Structure of the model

A system simulation model was developed. Allore et al.; (1999) demonstrated that discrete event mastitis models that accurately represent the biology of the system produced the most realistic output from the range of models examined.

In order to accurately represent the biology of mastitis within commercial dairy herds individual cows within individual herds are the basic unit modelled. The model is dynamic; with a single day being the basic time unit. Each cow in each herd is advanced through the model on a daily basis. Stochasticity is required to represent natural variation as well as uncertain information. This is to be introduced through the use of probability distributions describing individual cow's infection, cure, spontaneous cure and pregnancy status. Herd population dynamics (such as heifer replacement rates, non mastitis culling rates) are also obtained from existing probability distributions of Australian data.

The production of the model requires the following:

1. Elucidation of infection and cure probabilities for each pathogen.

2. Derivation of cow SCC and production response equations for a given infection status, pathogen type and stage of lactation.

3. Collection and incorporation of decision algorithms representing herd dynamics and current management practices.

Source of data

Maffra Veterinary Centre in the Macalister Irrigation District of Victoria collected data used in the development of the model from two studies conducted between 1994 and 1997. One study was the subclinical mastitis treatment trial conducted by the author that has been described in Chapter two. The second study was a longitudinal survey of clinical mastitis incidence and pathogen type in commercial dairy farms conducted by Dr Alison Gunn and Dr Jakob Malmo. These two studies allowed accurate identification of infection status, survival and production of a large number of cows. This data allowed the development of SCC predictive equations based upon infection status, pathogen and duration of infection. Similar equations for cow production were also derived.

The quantitative effect of risk factors such as teat dipping, dry cow therapy and milking machine maintenance upon the risk of new infection, cure rates and survival was collected from international literature review. Algorithms that define the current industry drying off and culling practices methods were represented in the model.

Results

Flow chart of model logic

The cow was the unit of simulation. At a given time, a cow will be either infected or uninfected. Infected cows have three possible outcomes during each simulated time unit; they can become clinical, remain subclinical or cure. Transition probabilities for each event determine the likelihood of each status for the cow at the next time period. Uninfected cows have two possible outcomes during a simulated time unit; they can remain uninfected or can become newly infected. Newly infected cows may become clinical or remain subclinical. Transition probabilities also determine the likelihood of each status for the cow at the next time unit. Transition probabilities are different for each of the pathogens studied and are different for new infections and chronic infections. The basic model is presented schematically in Figure 6.

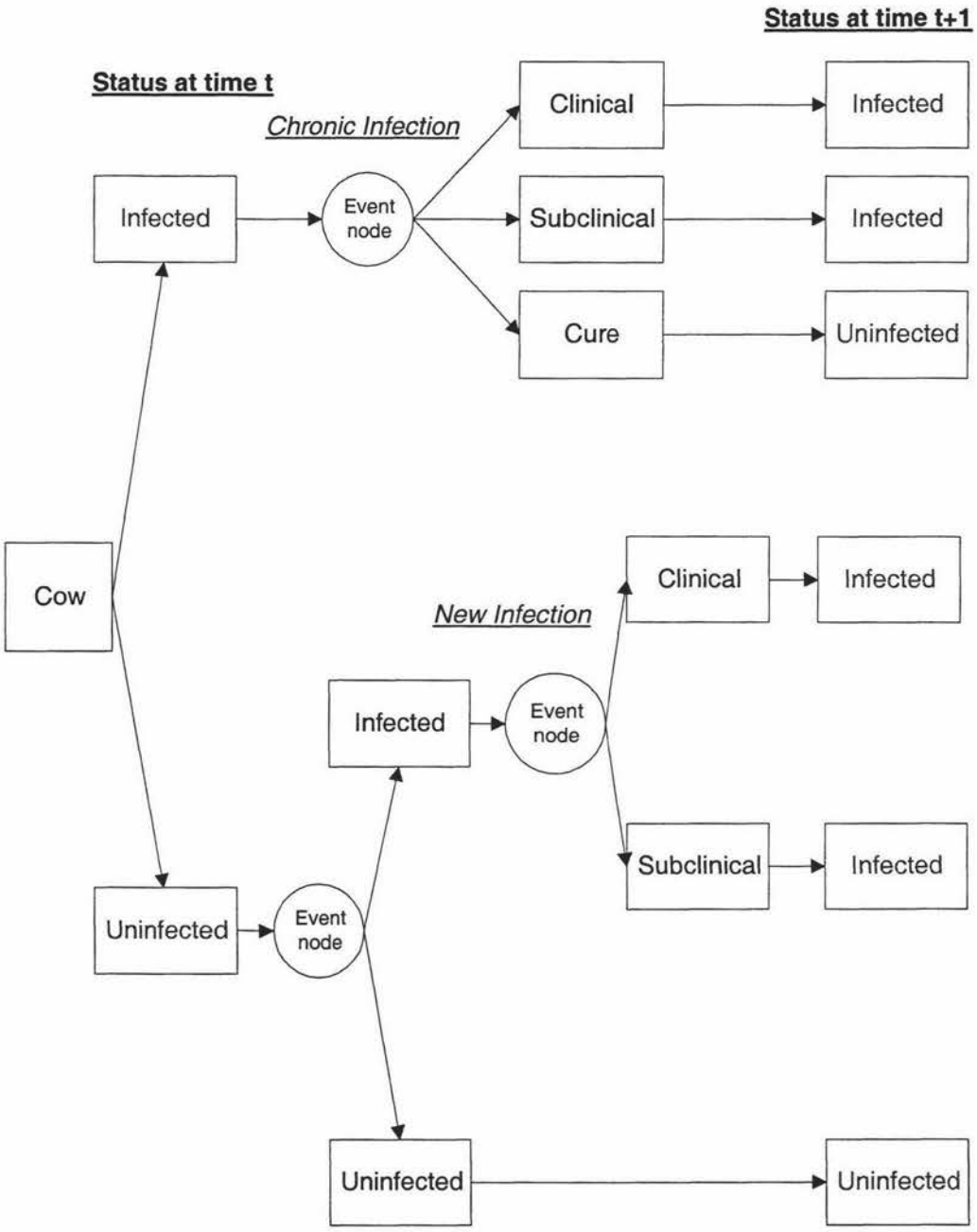

Figure 6: Schematic representation of the basic mastitis infection model logic

Derivation of transition probabilities

Domain experts examined the basic model flow chart and identified likely areas where risk factors would impact upon transition probabilities. These are summarised in Figure 7.

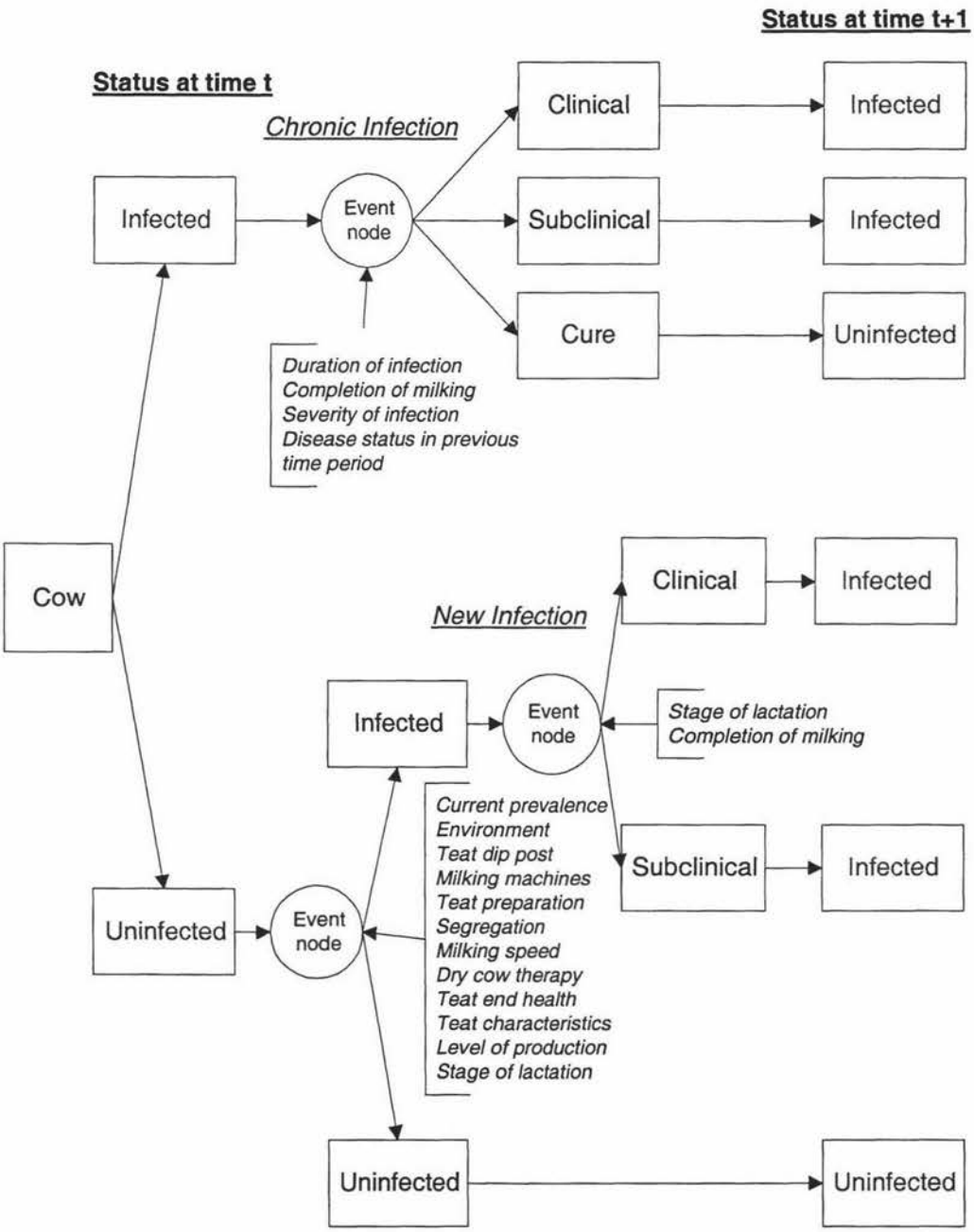

Figure 7: Sites of risk factor impact upon model transition probabilities

Literature review has indicated that there is poor agreement with respect to the probabilities of cows changing health status for any given combination of risk factors. Some variables also have not had their effect quantified in terms of risk (Allore et al., 1999). Problems have been identified in determining pathogen-specific probabilities for change of status. A number of approaches have been investigated in the past. These include use of chain-binomial probabilities (Lam et al., 1996) and logistic regression predictive equations

(Houben et al., 1993). All of the models studied have been insufficiently detailed to accurately study individual pathogens within individual herds.

The model building approach of Allore et al. (1999) was to use the logistic regression of Houben et al. (1993) to predict a baseline probability of infection for an individual cow per month. This regression predicts clinical disease occurrence per month only and is generic, as it does not specify pathogen type. Monthly probabilities for infection can be converted to daily probabilities by dividing the output by 30. The equation is given below

$$p = \frac{1}{1 + e^{-2.4239+0.07705*m+0.026267*l+0.154333*p+0.013525*pq-0.6349*cq+0.7814*dq}}$$

m = month of calving, l = lactation number, p = production level (1 = low, 2 = medium, 3 = high), pq = number of quarters infected in the previous lactation, cq=number of quarters infected up to and including the previous month, dq = number of quarters infected in current month.

Simulations with this equation did not direct support use within a simulation model for the following reasons:

1. The minimum probability of a clinical case per month was 0.166. This occurred when m=10, l=10, p=3, pq=4, cq=1 and dq=4. This is much higher than field observations would suggest.

2. The maximum probability of a clinical case per month was 0.980. This occurred when m=1, l=1, p=1, pq=1, cq=4 and dq=1. This is also much higher than field observations would suggest.

It will be necessary to modify the output from this equation if it is to be successfully incorporated into an Australian simulation model. The effect of preventive measures employed and the effect of individual pathogens on output will need to be examined. The relationship between clinical disease and infection will need to be examined further. The modification of the regression output to predict risk of new infection by factorial multiplication of the current output may result in probabilities greater than one. However the output from this equation has been successfully modified to allow incorporation into stochastic simulation models of mastitis (Allore et al., 1998 a). The best Australian estimate

of the size of the multiplier for conversion of the probability of clinical disease into the probability of infection is two. This is derived from the study of Hoare et al.,1982

If use of the logisitic regression equation of Houben et al., 1983 proves unsuitable, the use of baseline new infection probabilities that have been derived from field studies will be necessary. Baseline probabilities of new infection due to *S. aureus* and *S. uberis* were derived from the Australian study of Gunn et al., 1999. Incident clinical cases were aggregated into month of lactation and an exponential decay model of the form y = exp (A * (x - B)) was fitted to the data.

The model obtained for probability of a new clinical case of *S. aurues* is:

$$P(\text{new clinical case } S.\ aureus) = \exp(-0.275 * (\text{Month} + 19.784)); R^2 = 0.79$$

The predictive curve and 95% confidence intervals for probability of a new clinical case of *S. aurues* is given in Figure 8. The study of Hoare et al., 1982 indicated that 50% of infected cows develop clinical disease. Using this association, the probability of a new infection with *S. aureus* is given by the relationship:

$$P(\text{new infection } S.\ aureus) = 2 * (\exp(-0.275 * (\text{Month} + 19.784)))$$

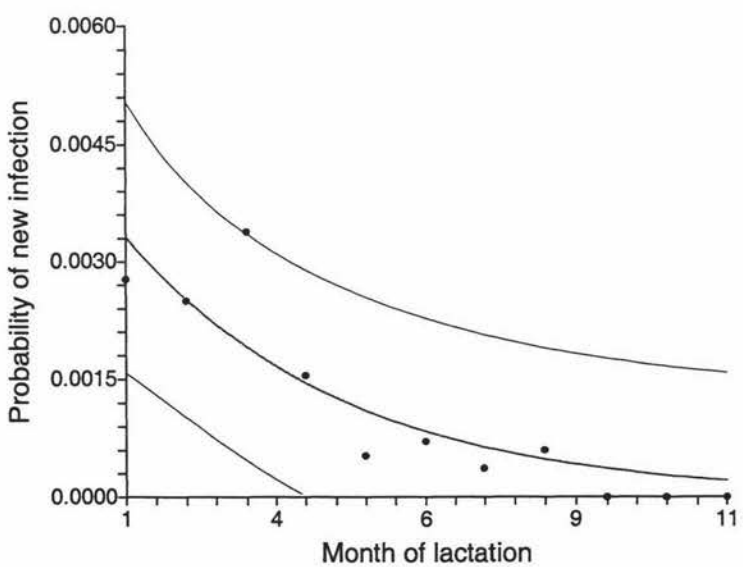

Figure 8: Regression curve of the probability of an incident clinical case of *S aureus* mastitis versus month of lactation with associated 95% confidence intervals

The model obtained for probability of a new clinical case of *S. uberis* is:

$$P \text{ (new clinical case } S. \textit{uberis}) = \exp(-1.345 * (\text{Month} + 2.353)); R^2 = 0.97$$

Using that only 50% of new infections become clinical (Hoare et al., 1982), the final model that predicts new infection with *S. uberis* is:

$$P \text{ (new infection } S \textit{ uberis}) = 2 * (\exp(-1.345 * (\text{Month} + 2.353)))$$

The predictive curve and 95% confidence interval for probability of a new clinical case of *S. uberis* is given in Figure 9.

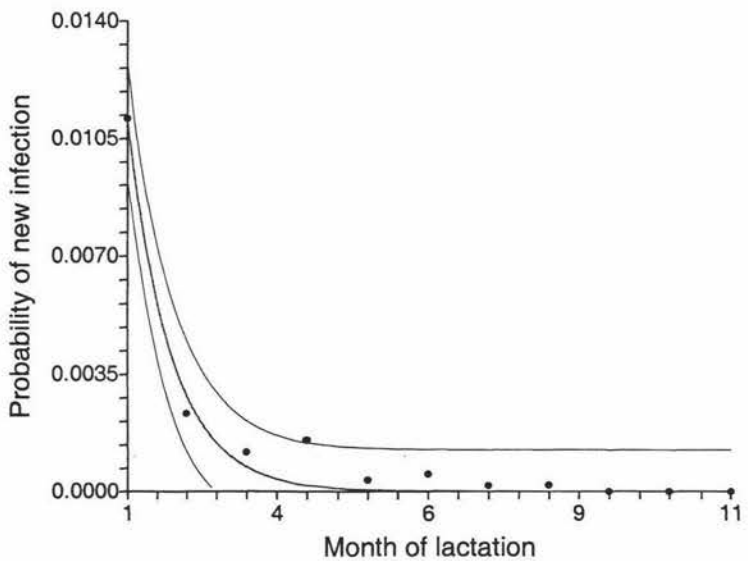

Figure 9: Regression curve of the probability of an incident clinical case of *S uberis* mastitis versus month of lactation with associated 95% confidence intervals

Both the new infection model of Houben et al.,1983 and the new infection predictive model derived from the data of Gunn et al., 1999 will be tested in the model building stage. The most suitable new infection predictive equations after model verification and validation studies will be incorporated into the final version.

Baseline probabilities for infection with specific pathogens for the period from the start of the dry period to immediately after calving in cows that are treated with dry cow antibiotic and in cows that are not treated with dry cow antibiotics are presented in Table 27 and

Table 28. Relative risk of new infection comparing dry cow treated and untreated cows is presented in Table 29 (Allore et al., 1998 a).

Table 27: Transition probabilities for infection from the start of the dry period to parturition without dry cow antibiotic therapy

	Infection status immediately post calving			
Infection status at dry-off	Uninfected	*S. agalactiae*	*S. uberis*	*S. aureus*
Uninfected	0.76	0.02	0.09	0.02
Infected				
S. agalactiae	0.32	0.64	0.02	0.02
S. uberis	0.50	0.00	0.47	0.00
S. aureus	0.20	0.00	0.04	0.71

Table 28: Transition probabilities for infection from the start of the dry period to parturition following dry cow antibiotic therapy

	Infection status immediately post calving			
Infection status at dry-off	Uninfected	*S. agalactiae*	*S. uberis*	*S. aureus*
Uninfected	0.95	0.00	0.02	0.00
Infected				
S. agalactiae	0.89	0.00	0.04	0.02
S. uberis	0.70	0.00	0.20	0.00
S. aureus	0.60	0.00	0.04	0.36

Table 29: Relative risk of infection from the start of the dry period to parturition for cows treated with dry cow antibiotic therapy compared to untreated cows

	Infection status immediately post calving			
Infections status at dry-off	Uninfected	*S. agalactiae*	*S. uberis*	*S. aureus*
Uninfected	1.25	0.00	0.22	0.00
Infected				
S. agalactiae	2.78	0.00	2.00	1.00
S. uberis	1.40	1.00	0.43	1.00
S. aureus	3.00	1.00	1.00	0.51

In the Allore et al., 1998 model, the baseline probability of Houben et al., 1983 was modified according to the combination of the 23 model risk factors represented in the simulation. The modified probability obtained from this process was then used in the simulation. Infection pressure was one factor used to adjust baseline probabilities of infection. The mechanism of exposure differs between the contagious and environmental pathogens. Spread of contagious mastitis pathogens within a herd has been shown to fit a chain-binomial model (Lam et al., 1996). Contagious pathogen infection probabilities will change depending upon infection pressure (prevalence) within the herd. If a homogenous mixing structure is used, the probability of exposure to *S. aureus* is:

$$\text{p (exposure)} = \frac{\text{number cows infected}}{\text{total number of cows}}$$

The simulation of Allore et al. (1999) had initiation prevalence values of 14.7%. This value was used as a scaling factor for exposure on risk of new infection as follows:

$$\text{risk ratio (exposure)} = \left(\frac{\text{number cows infected}}{\text{total number cows}} \right) / 0.147$$

The study of Gunn et al., 1999 had a study prevalence of clinical disease per year of 2.56% for *S. aureus.* Using the association of Hoare et al., 1982, this equates to a prevalence of infection per year of 5.12% for *S. aureus.* Using the identical methodology to above, the scaling factor for the *S. aureus* new infection predictive equation is:

$$\text{risk ratio (exposure)} = \left(\frac{\text{number cows infected}}{\text{total number cows}} \right) / 0.0512$$

Exposure to an environmental pathogen is independent of herd prevalence. Exposure risk is constant. This is of the form

$$\text{p (exposure)} = \text{p (}S.\ uberis\text{)}$$

Allore et al. (1999) estimated that the risk of environmental infection with *S. uberis* in cows previously uninfected after calving was 0.09 if no dry cow therapy was applied and 0.02 if dry cow therapy had been used. Therefore, the risk ratio becomes 0.22 (p = 0.02/0.09) if dry cow therapy is applied. Ongoing lactation risk of new infection with *S. uberis* was not

given. It is likely that these probabilities will have to be modified during the simulation phase to suit the Australian management system of seasonal calving and non-housed cattle.

The effects of exposure are modified through preventive methods such as teat dipping, dry cow therapy and milking machine maintenance. The final probability of new infection becomes:

$$P(\text{infection}) = 2 * P(\text{clinical disease}) * \text{risk ratio (exposure)} * (1 - \text{preventives})$$

Where P (clinical disease) = logistic regression output of Houben et al. (1993), or the new infection probabilities of Gunn et al., (1999)

The output from this equation will need to be validated before further incorporation into the simulation model architecture.

The effect that each risk factor has upon transition probabilities according to domain experts was examined by literature review. Many risk factor probabilities have not been quantified. Some risk factor studies have resulted in conflicting conclusions and probabilities. Model verification, validation and simulations will be required to clarify the role of each risk factor. A brief summary of risk factor relationships is given in Table 30.

Table 30: Quantification of risk factor probabilities as obtained from international literature review

Model node	Control point	All	S aureus	S uberis
New Infection	Environment	Risk of new infection in heifers at calving = 0.45. New infection rate in dry period = 3.8% if no dry cow therapy and 2.1% if dry cow therapy applied (Mein at al., 1990)		Contribute around 65% of clinical cases - 20 cases/100 cows year. Leaking milk before calving increases risk of clinical disease in lactation (RR = 1.8). New infection rate per 1000 quarter milkings = 0.53 if teats are exposed (Hutton et al., 1991)
New Infection	Teat dipping (post)	Very significant reduction in new infection risk. 2.2 fold reduction in SCC compared to DCT (Gill et al., 1990)	Teat dipping reduces relative risk of new infection. For epidemics, RR= 0.14; (86% efficacy) and for endemic situations RR = 0.46 (55% efficacy) (Lam et al., 1996)	Post milking teat dipping has no effect upon environmental infections (Schukken at al., 1991)
New Infection	Milking machines	Overmilking leads to a 60% increase in NIR for all pathogens (Hogeveen et al., 1995)	With good machines and 100% exposure to s aureus NIR range is= 0.00004.2-8.3 per quarter milking. Regular cleaning machines reduces prevalence by 10%. Other effects not quantified (Hutton et al., 1991)	
New Infection	Teat preparation		Disinfection prior to use of an IM tube produced a 27% reduction in s aureus prevalence (Gill et al., 1990)	Pre dipping is highly effective (not quantified) (Gill et al., 1990)
New Infection	Segregation		Effective reduction in NIR. (not quantified) (Hutton et al., 1991)	

Model node	Control point	All	S aureus	S uberis
New Infection	Milking speed	Odds ratios for NIR for high, average and low producers are 1.25, 1.0, and 0.75. (Grohn et al., 1995)	High flow rate cows have higher NIR's (RR = 12 when comparing quarters with flow rates exceeding 1.6l/min to those with flow rates under 0.8l/min) (Grindal et al., 1991)	
New Infection	Dry cow therapy	6.4% increase in clinical cases in subsequent lactation after DCT (Storper et al., 1981). DCT reduces risk of new infection in subsequent lactation (RR = 0.68) (Nickerson, 1987). NIR from dry off to calving - 8.4-19.6% in DCT cows (Mein at al., 1990)	10% NIR if infected quarters only are treated. 2.3% NIR if all quarters are treated. Blanket DCT reduces risk of new infection (RR= 0.23) (Mein at al., 1990)	Dry period NIR = 1.5% if no DCT applied. This reduces to 0.4% if DCT applied (RR = 0.27) (Mein at al., 1990).
New Infection	Teat end health	Damaged teat ends have a 1.5-fold increase in RR of clinical episode for all pathogens (Elbers et al., 1998)	Studies that injected S aureus through the teat canal had NIR of 100% (Woolford et al., 1983)	Damaged teat ends result in a 2-fold increase in RR of clinical disease due to E coli. (Elbers et al., 1998)
New Infection	Stage of lactation	Proportion of clinical cases in the first 35 days of lactation (Lactations 1-5) is .60,.36,.31,.30,.28. If cows experience two clinical episodes during lactation there is a 75% probability of another occurrence during the same lactation (Morse et al. 1987). Recurrence times fit a gamma distribution, with 30% occurring within 1 month and 44% within 2 months (Allore et al., 1999)	Around 50% of new cases become clinical assuming two infected quarters per cow (Hoare et al., 1977)	
New Infection	All control	Preventive measures result in a 50% decrease in NIR. No synergy between preventives has been observed. DCT, teat dipping and milking machine servicing result in a 50% decrease in NIR risk compared to use of no preventives (Allore et al., 1998 c).	The 5-point plan has decreased the prevalence of contagious pathogens by 90% (17%->1%). Other studies demonstrated a halving of the number of quarters infected following use of the 5-point plan (Hillerton et al., 1995)	5-point plan has had no effect environ mast. 65% cases are now environmental; the clinical case rate is unchanged (Hillerton et al., 1995)

Model node	Control point	All	S aureus	S uberis
Chronic Infection – cure	Duration of infection	50% self cure rate by 14 days to minor paths. 70% cure rate if treated (Timms et al., 1983). Cure rate decreases from 82% in 2-3 year olds to 60% in 5-10 year olds. 76% of cases at calving and 70% of cases at mid lactation were in cows infected in the previous lactation (Mein at al., 1990)	63% cure rate to treatment in young cows. Cure rate range 0-60%; 70% if less than 2 months infected and 18% if more than 12 months infected (Storper et al., 1981. Klastrup, 1987). 65% cure rate to DCT (Sol et al., 1994. Osteras et al., 1994). Spontaneous dry period cure rate is 34%. (Allore et al., 1998 b). Average duration of infection for cured cows is 7 days. The average duration of infection for uncured cows is 135 days (Allore et al., 1998 b).	90% of strep infections are cured by treatment (Storper et al., 1981). The spontaneous cure rate is 59% (Allore et al., 1998 b)
Chronic Infection – cure	Vaccination		Vaccine studies have demonstrated a reduction in odds of disease (OR = 0.5) (Calzolari et al., 1997)	
Chronic Infection – cure	Severity of infection	Average GMSCC prior to a clinical episode are: mild – 290,000, severe – 247,000. Average GMSCC 30 days after a clinical episode are: mild - 409,000, severe – 592,000. This difference persists all lactation. (Dohoo et al., 1984)	Cure rate to DCT decreases as SCC increases. p(cure cow) = $1/(1+e^{(-5.57 +3.35 \log SCC - 0.34 \log SCC^2 - 0.19 Age (years) -0.56 (more than 1 quarter infected))}$. The cure rate halves for every extra quarter infected. (Sol et al., 1994)	
Chronic Infection – cure	Disease status at t-1	High SCC cows have a 28.6% relapse rate in the current lactation. New clinical cases have a 37.5% relapse rate in the current lactation (Timms et al., 1983). 50% of clinical cases occur in high SCC cows (Woolford et al., 1983).	30% of clinical heifers and 45% of clinical cows will have a second case during the current lactation. 30% of heifers and 60% of cows will have a third episode (Morse et al. 1987)	

Model node	Control point	All	S aureus	S uberis
Chronic Infection – cure	Stage of lactation	Probability (dry period infection) = 0.059 + 0.31*(prevalence of Infected cows) (Natzke et al., 1974). The range of dry period new infections is 2% to 14.6%. The spontaneous dry period cure rate is 60%. (Lescourret et al., 1995)	20% of infected cows in the previous lactation will experience a clinical episode in the current lactation despite DCT (Morse et al. 1987)	
SCC – clinical	Duration of infection	Average duration of clinical disease is 6.6 days (Morse et al., 1987)		
SCC – clinical	No. quarters infected		Farms with 100% prevalence (cows) had an average SCC of 739,000 and an average of 1.72 infected quarters per cow (Woolford et al., 1983)	
SCC – subclin	Stage of lactation	Once production falls below 5 litres per day, SCC will increase by more than 50,000 cells/week. This is irrespective of infection status (Lacy-Hulbert et al., 1995)		
SCC – subclin	Duration of infection	6% cows have SCC less than 400,000 at treatment, 47% are less than 400,000 by 7 days and 55% are less than 400,000 by 14 days. (Timms et al., 1983). Cure does not reduce SCC significantly for the remainder of the lactation (Seymour et al., 1989).	CMT level, number of quarters infected, lactation number and stage of lactation have negative influences upon cure rates. This was not quantified (Miller, 1974)	
SCC – subclin	Disease status at t-1	All pathogens produce a similar SCC response following infection (Storper et al., 1981). You cannot predict causative pathogen based on SCC (Kirk et al., 1996 b).		There is a significant lowering of SCC within 1 month of infection. This indicates that the self cure rate is 75% (Kirk et al., 1996 b)

Model node	Control point	All	S aureus	S uberis
SCC – subclin	No. quarters infected	Uninfected cows increase an average of 80,000 cells/ml over the duration of the lactation (Sheldrake et al., 1983)		

The method of incorporation of the relationships described in Table 30 into computer model construction is briefly described in Table 31. The model nodes were identified from model flow chart construction and are presented in Figure 6 and Figure 7.

Table 31: Likely methods of incorporation of risk factor probabilities as obtained from international literature review into model architecture

Model node	Control point	All	S aureus	S uberis
New Infection	Environment	New infection probability estimate. Risk factor weighting for use of dry cow therapy		Risk factor weighting for new infection due to leaking milk. Estimate of new infection rate
New Infection	Teat dipping (post)	Risk factor weighting for effect of teat dipping upon risk of new infection	Risk factor weighting for effect of teat dipping upon risk of new infection	Risk factor weighting for effect of teat dipping upon risk of new infection
New Infection	Milking machines	Risk factor weighting for effect of milking machines upon new infection rate	Risk factor weighting for effect of milking machines upon new infection rate	
New Infection	Teat preparation		Risk factor weighting for effect of pre dipping on new infection rate	Risk factor weighting for effect of pre dipping on new infection rate
New Infection	Segregation		Risk factor weighting for effect of segregation upon new infection rate	
New Infection	Milking speed	Risk factor weighting for effect of milking speed upon risk of new infection	Risk factor weighting for effect of milking speed upon risk of new infection	
New Infection	Dry cow therapy	Risk factor weighting for effect of dry cow therapy upon risk of new infection in the next lactation	Risk factor weighting for effect of dry cow therapy upon risk of new infection in the next lactation	Risk factor weighting for effect of dry cow therapy upon risk of new infection in the next lactation

Model node	Control point	All	S aureus	S uberis
New Infection	Teat end health	Risk factor weighting for effect of teat end damage upon risk of new infection	Risk factor weighting for effect of teat end damage upon risk of new infection	Risk factor weighting for effect of teat end damage upon risk of new infection
New Infection	Stage of lactation	Distribution pattern of clinical cases versus stage of lactation	Probability of recurrence of clinical episode during lactation	
New Infection	All control	Summary of total effect of control strategies upon risk of new infection. Provides guidelines to multiplicity (interaction) between individual risk factor probabilities	Summary of total effect of control strategies upon risk of new infection. Provides guidelines to multiplicity (interaction) between individual risk factor probabilities	Summary of total effect of control strategies upon risk of new infection. Provides guidelines to multiplicity (interaction) between individual risk factor probabilities
Chronic Infection – cure	Duration of infection	Duration of infection distribution and probability of recurrence during a lactation	Duration of infection distribution, probability of recurrence during a lactation and the spontaneous and treatment cure rates during lactation	Spontaneous and treatment cure rates during lactation
Chronic Infection – cure	Vaccination		Risk factor weighting of the effect of vaccination on duration of disease and risk of recurrence	
Chronic Infection – cure	Severity of infection	Effect of severity of infection upon SCC output of cows	Quantifies the effect of severity of infection as measured by SCC upon probability of cure	
Chronic Infection – cure	Disease status at t-1	Quantification of the risk of recurrence and cure rates of chronically infected cows during lactation	Quantification of the risk of recurrence and cure rates of chronically infected cows during lactation	
Chronic Infection – cure	Stage of lactation	Spontaneous cure rates in dry period and effect of prevalence upon risk of another clinical episode during the subsequent lactation. Outline of variability in risk of new infection between herds	Quantification of the risk of another clinical episode in the subsequent lactation following infection in the preceding lactation	
SCC – clinical	Duration of infection	Estimate of the average duration of clinical disease		

Model node	Control point	All	S aureus	S uberis
SCC – clinical	No. quarters infected		Estimate of the average number of infected quarters per cow in high prevalence and low prevalence herds	
SCC – subclin	Stage of lactation	Effect of stage of lactation upon SCC for uninfected and infected cows		
SCC – subclin	Duration of infection	Distribution of SCC output of clinical cows based upon duration of infection	Effect of risk factors upon the cure rate probabilities of infected cows	
SCC – subclin	Disease status at t-1	Distribution of SCC output of cows following infection based upon causative pathogen		Distribution of SCC output of infected cows after infection
SCC – subclin	No. quarters infected	Distribution of SCC output of uninfected cows over the duration of a lactation		

Modelling the effect of infection upon somatic cell count

A regression equation to predict the SCC of an individual cow for a given number of days in milk, infection status, clinical disease status and pathogen status was required. The SCC response of cows after calving is curvilinear. The average SCC of uninfected cows versus days calved (SOL) and the regression equation using SOL as the predictor variable is given in Figure 10. This regression is given below ($R^2 = 0.90$).

$$\text{Pred SCC} = 2.2203 - 0.5524 * \log10\,(\text{SOL}) + 0.0048 * (\text{days}) - 3.1\text{E-}06 * (\text{SOL}^2)$$

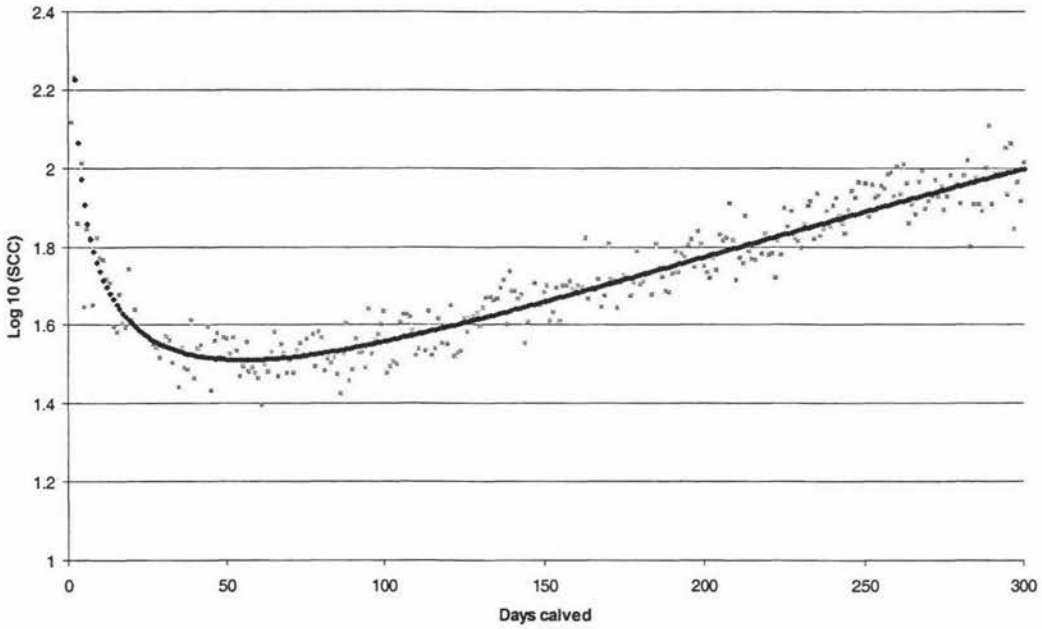

Figure 10: Log 10 average SCC and regression line versus days calved (SOL)

Herd test data has a repeated measures component; most cows have five herd test records per lactation. Simple multivariable regression techniques are inappropriate due to the autocorrelation between errors. A mixed multivariable regression model was developed using Mixreg 1.2 (Hedeker and Gibbons (1993)). The variables presented to the model included an intercept, SOL, Pred SCC, *S. aureus* status, *S. uberis* status, clinical disease status, subclinical disease status, duration of infection (in days), and interaction terms between *S. aureus* status and duration of infection (Saur * Dur) and *S. uberis* status and duration of infection (Sub * Dur). Results are given in

Table 32. Maximum Log Likelihood was –3438.7, residual variance was 0.052 and autocorrelation between log 10 SCC within cows was 0.57.

Table 32: Mixed regression coefficient estimates for SCC predictive equation

Variable	Coefficient	Stand. Error	Z value	P-value
SOL	-0.006	0.001	-5.688	0.000
Intercept	0.482	0.043	11.131	0.000
Predicted SCC	0.699	0.030	22.994	0.000
S. uberis	-0.342	0.071	-4.777	0.000
Clinical	1.276	0.067	18.846	0.000
Subclinical	1.481	0.056	26.329	0.000
Duration	-0.003	0.000	-14.443	0.000
Saur * Dur	0.001	0.000	5.018	0.000
Sub * Dur	0.002	0.0003	6.652	0.000

The final SCC predictive model is given below.

LogSCC = 0.482 + 0.700 * (Pred.SCC) - 0.007 * (SOL) - 0.343 * (*S. uberis*) + 1.276 * (clinical) + 1.482 * (subclinical) – 0.004 * (duration) + 0.002 * (Saur*Dur) + 0.002 * (Sub*Dur)

Stochasticity in individual cow SCC and production response was modelled through the use of control variates (Law and Kelton., 1991). Control variates randomly assign an individual a variation value. Monte-Carlo sampling from the distribution of data residuals chooses this value. The chosen individual variation value is then added to the individual regression equation estimates. The residuals for SCC were studied. The first SCC between day 30 and day 100 of lactation was used for each cow, as the SCC in this time period was the most stable for most cows. The high SCC's that occur immediately after calving and towards the end of lactation were not examined. The regression residuals obtained from observations made between day 30 and day 100 are representative of the spread in SCC for all cows at any given stage of lactation. Residuals from these observations had a mean of 0 and a standard deviation of 0.360. (N (0, 0.360)). The stochastic SCC predictive model becomes:

LogSCC = N (0, 0.360) + 0.483 + 0.700 * (Pred.SCC) – 0.007 * (Time) – 0.343 * (Suberis) + 1.276 * (Clinical) + 1.482 * (Subclinical) – 0.004 * (Duration) + 0.002 * (Saur*Dur) + 0.002 * (Sub*Dur)

This equation produced outputs with similar range, maximum, minimum, mean SCC and standard deviation as was found in the study data.

Modelling the effect of infection upon production

There have been many studies on the effects of infection status upon production. These have been discussed in Chapter one. Regression equations for daily milk, fat and protein production were generated from the data.

Litres

Average daily production (litres) per cow versus stage of lactation was modelled using regression analysis. The results are presented in Figure 11.

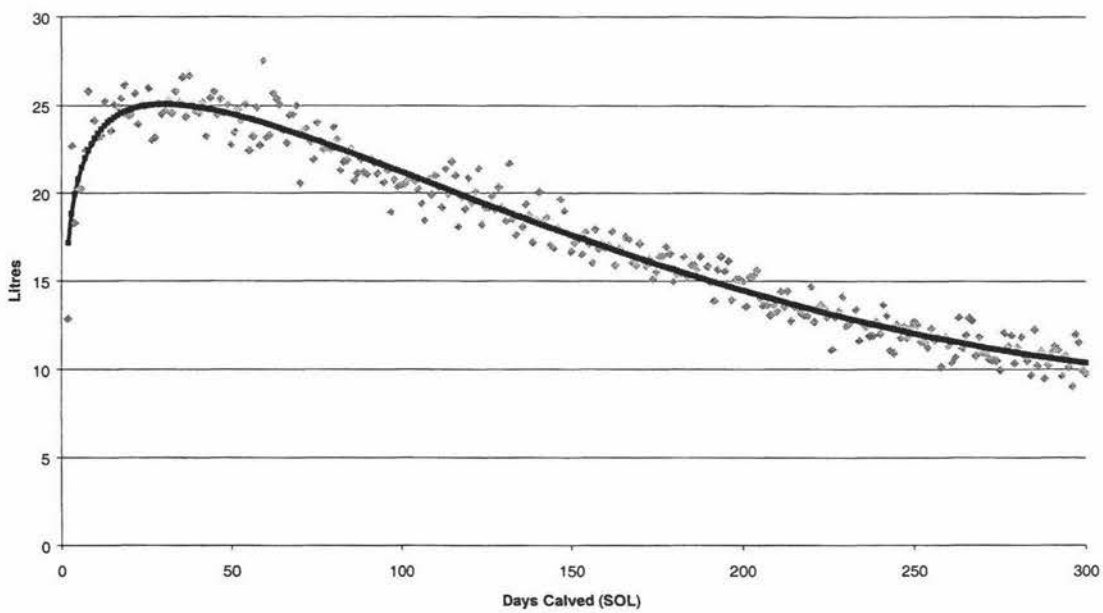

Figure 11: Average daily litres milk and regression line versus days calved

The regression analysis output for predicted litres is presented in

Table 33. The corresponding regression equation obtained was:

Predicted Litres (PredL) = 14.353 + 10.331 * Log10(SOL) - 0.158 * (SOL) + 0.0002 * (SOL^2)

This regression (PredL) was forwarded to the multivariable mixed regression model.

Table 33: Regression coefficient estimates of predictive equation for daily milk volume (litres) only including days in lactation as a predictor variable

Variable	Coefficient	Stand. Error	Z Value	P-value
Intercept	14.353	0.605	23.741	2.09E-70
Log10 (SOL)	10.331	0.531	19.451	8.4E-55
(SOL)	-0.158	0.006	-25.078	4.14E-75
$(SOL)^2$	0.0002	1.43E-05	13.741	1.51E-33

Mixed regression analysis output was generated. Significant variables in the final model were herd, heifer, second calver and predicted litres (PredL). Log likelihood was –2803.5 and residual variance was 5.83. Regression analysis output is given in Table 34.

Table 34: Mixed regression coefficient estimates of predictive equation for daily milk volume (litres)

Variable	Estimate	Stand. Error	Z Value	P-value
Herd	-2.002	0.526	-3.803	0.000
Heifer	-2.414	0.203	-11.894	0.000
2^{nd} Calver	-0.812	0.221	-3.700	0.000
PredL	1.061	0.006	179.106	0.000

Infection status variables (clinical disease, subclinical disease, pathogen, duration of infection and appropriate interaction terms) were offered to the model. No infection status variables were significant. This finding supports much of the recent literature on production loss following infection (see Chapter 1). The final multivariable regression is:

Litres = 1.06139 * PredL - 2.00247 * Herd - 2.41384 * Heifer - 0.81233 * 2^{nd} Calver

Milk production varies between individuals and between herds. To make the model mirror this variation control variates were introduced to the model. The residuals for daily litres were examined. Within cow residuals had a mean of 0 and a standard deviation of 5.88 litres; (N (0, 5.88)). Spreadsheet simulations involving the addition of a random term

sampled from the N (0, 5.88) distribution resulted in output with similar range, maximum, minimum, mean and standard deviation compared with the original data. Addition of this term allowed the regression to cater for individual variation. The stochastic SCC predictive model equation became:

Litres = N (0, 5.88) + 1.062 * PredL - 2.002 * Herd - 2.414 * Heifer - 0.812 * 2nd Calver

Between herd variation was modelled using a similar technique. The original data was examined on a herd basis and the between herd average cow production distribution described. Between herd average cow production had a mean of 0 and a standard deviation of 4.98 litres; (N(0,4.98)). A term for herd variation was added to the multivariable regression model as follows:

Litres = Herd {N (0, 4.98)} + Cow {N (0, 5.88)} + 1.062 * PredL - 2.002 * Herd - 2.414 * Heifer - 0.812 * 2nd Calver

The inclusion of the herd effect into the final equation will support the use of the final mastitis model in simulations involving a random selection of herds. The modelling of specific herds with a given herd production level will require that the random herd effect be bypassed. This would occur through the assignment of a specific herd production effect (eg 4.2 litres). Programming will need to ensure that the final architecture will have these dual capabilities.

Fat

Daily fat production per cow was modelled using a similar method as for daily litre production. The average daily fat production per cow was regressed against stage of lactation. The resultant predicted fat percentage (PredF%) regression was then sent forward to the multivariate stage. The average daily fat percentage and the predicted fat percentage regression line are given in Figure 12.

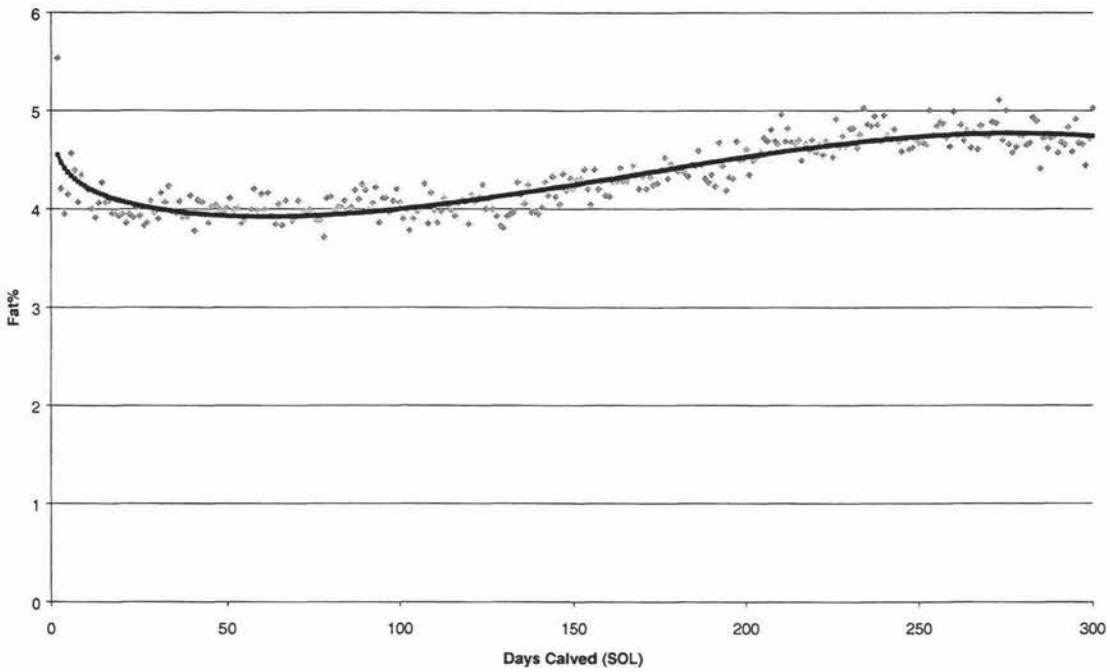

Figure 12: Average daily fat percentage and regression versus days calved

The predicted daily fat percentage (PredF%) versus days calved (SOL) regression analysis output is given in Table 35. The final univariate regression equation for prediction of daily fat percentage was:

$$PredF\% = 4.679 - 0.413 * Log10\,(SOL) - 0.004 * (SOL) + 6.95E\text{-}05 * (SOL)^2 - 1.5E\text{-}07 * (SOL)^3$$

Table 35: Regression coefficient estimates of predictive equation for daily milk fat percentage only including days in lactation as a predictor variable

Variable	Coefficient	Stand. Error	Z Value	P-value
Intercept	4.679	0.114	41.131	5.7E-124
Log10 (SOL)	-0.413	0.124	-3.318	0.001
SOL	-0.004	0.003	-1.540	0.124645
$(SOL)^2$	6.95E-05	1.4E-05	4.951	1.25E-06
$(SOL)^3$	-1.5E-07	2.61E-08	-5.614	4.59E-08

Significant variables following multivariable (mixed) regression analysis were herd, SOL, Heifer, predicted fat% (PredF%) and litres. No infection status variables were significant. Once again this supports previous work indicating that infection does not have a significant effect upon production. Mixed regression output is given in Table 36.

Table 36: Mixed regression coefficient estimates of predictive equation for daily milk fat percentage

Variable	Coefficient	Stand. Error	Z Value	P-value
Herd	-0.154	0.044	-3.507	0.000
SOL	-0.014	0.001	-10.795	0.000
Heifer	-0.066	0.024	-2.682	0.007
PredF%	1.148	0.010	113.097	0.000
Litres	-0.017	0.001	-14.7122	0.000

The final multivariable regression equation is:

Fat% = 1.148 * (PredF%) - 0.154 * (Herd) – 0.014 * (SOL) – 0.066 * (Heifer) – 0.017

Herd identifier was offered to the model for prediction of fat percentage to allow inclusion of a herd effect. The significance of the herd term in the final model supports the field observations of variation in the average fat percentage between herds. Variability between herds can now be modelled through use of a random number generator.

No between cow variability term was included as there were no important infection status terms on cow production output in the final model. Inclusion of a between cow variability term would add unnecessary complexity to the model.

Protein

Daily protein production per cow was modelled using the same method as for daily litre and fat percentage production. The average daily protein production per cow was regressed against stage of lactation. This regression model (predicted protein percentage (PredP%)) was then used as the basis for the multivariate stage. The average daily protein percentage and the predicted fat percentage regression line are given in Figure 13.

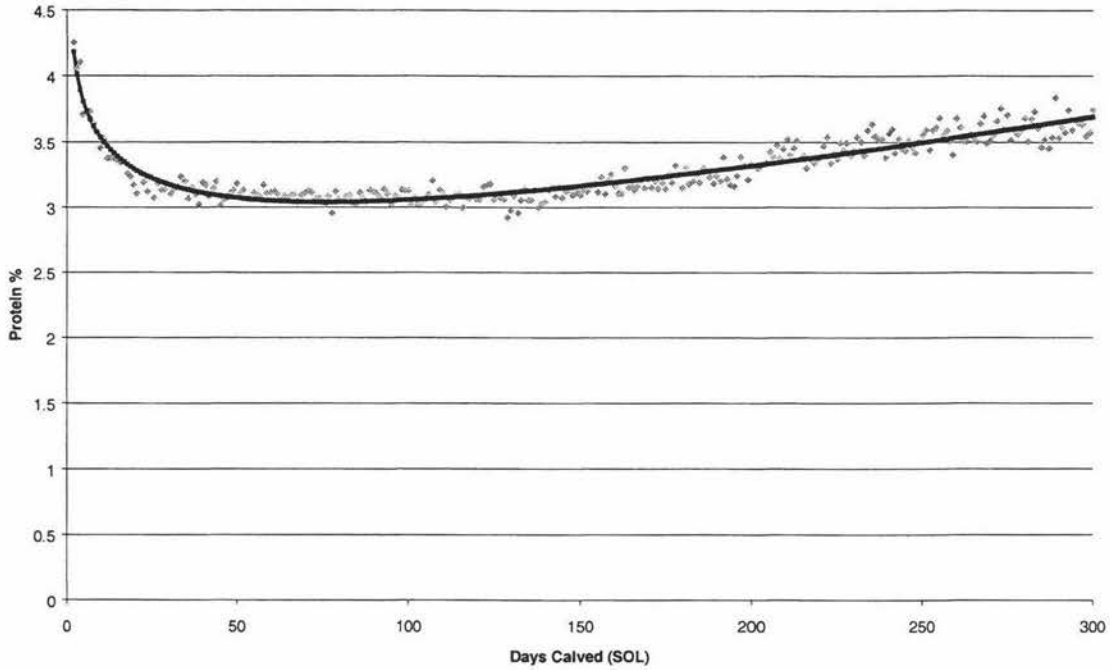

Figure 13: Average observed daily protein percentage and predicted regression line versus days calved

The regression output for predicted protein percentage versus stage of lactation is given in Table 37.

Table 37: Regression coefficient estimates of predictive equation for daily milk protein percentage only including days in lactation as a predictor variable

Variable	Coefficient	Stand. Error	Z Value	P-value
Intercept	4.469	0.046	98.262	2E-227
Log10 (SOL)	-0.990	0.040	-24.790	4.2E-74
(SOL)	0.006	0.000	12.151	8.1E-28
$(SOL)^2$	-5.4E-07	1.1E-06	-0.503	0.615

The final univariate model is:

$$PredP\% = 4.469 - 0.990 * Log10\,(SOL) + 0.006 * (SOL) - 5.4E\text{-}07 * (SOL)^2$$

The significant variables following the multivariable stage were predicted protein percentage (PredP%), stage of lactation (SOL), heifer, second calver, litres and fat percentage. Fat and protein percentages are correlated amongst farms, thus the existing fat percentage was offered to the model. This was a significant variable. In the simulation

stage the output from the fat percentage regression will be offered to the protein percentage predictive model as a substitute for actual fat percentage.

A herd variable was offered to the protein model in an identical manner to the fat percentage predictive model; however this was not a significant term in the final model. It is likely that inclusion of the predicted fat percentage variable within the protein model is also including the effect of herd upon predicted protein percentage. Final protein model mixed regression output is given in Table 38.

Table 38: Mixed regression coefficient estimates for daily protein % predictive equation

Variable	Coefficient	Stand. Error	Z Value	P-value
SOL	-0.004	0.000	-7.961	0.000
2nd Calver	-0.030	0.011	-2.737	0.006
Heifer	-0.079	0.010	-7.661	0.000
PredP%	0.814	0.007	112.818	0.000
Litres	-0.003	0.000	-6.215	0.000
Fat%	0.174	0.004	43.965	0.000

The final multivariable regression equation is:

Protein % = 0.814 * (PredP%) - 0.004 * (SOL) – 0.079 * (Heifer) - 0.030 * (2nd Calver) – 0.003 * (Litres) – 0.174 * (Fat %)

Development of decision algorithms

The major decision algorithms to be included in the final model are for culling and drying off, herd age strata composition and cow age strata survival rates to the next lactation.

Culling and drying off algorithms reflect current Australian recommendations (Brightling et al., 1998). A cow is culled if she experiences a third clinical episode within the same lactation. Ideally, this should be based upon quarter recurrence rates within lactation (Brightling et al., 1998), however the model to be developed is based upon the cow and not the quarter as the basic unit. It is not expected that generalisation of this algorithm from the quarter level to the cow level will impact significantly on model output, but output will be examined in the model verification and validation stage for anomalies relating to this generalisation.

A cow is treated and dried off if she experiences a clinical episode within one month of the end of lactation. Cows are dried off if their daily production is less than six litres.

The author has previously derived herd age strata structure and survival probabilities from Australian herd testing data. This is summarised in Table 39.

Table 39: Survival probabilities and herd age strata structure for all 1998 herd testing farms of the Maffra Herd Improvement Co-operative

Age at calving (years)	Probability of surviving the previous season	Cumulative survival probability of previous year(s)	Proportion of milking herd
2	1.00	1.00	0.18
3	0.85	0.85	0.15
4	0.86	0.73	0.13
5	0.89	0.65	0.12
6	0.87	0.56	0.10
7	0.85	0.48	0.09
8	0.82	0.39	0.07
9	0.78	0.30	0.05
10	0.78	0.24	0.04
11	0.71	0.17	0.03
12	0.63	0.10	0.02
13	0.56	0.06	0.01
14	0.51	0.03	0.01
15	0.51	0.01	0.00

Discussion

Production regression equations accurately mirror the Australian production system and are suitable for use in simulation. The estimation of infection transition probabilities is likely to be an iterative process. The only predictive model for new infection risk in the international literature is the logistic regression model of Houben et al. (1993). This model predicts risk of clinical disease only, is generic (not pathogen specific), does not distinguish between new infections and relapses occurring in chronically infected cows and was derived from European data. A pathogen specific model that predicts risk of new infection is essential for production of an accurate simulation model, however successful modification and incorporation of this regression equation into a working mastitis predictive model has

occurred in the USA (Allore et al., 1999). The model building stage will determine if this regression equation can be modified to accurately predict the Australian system.

Differences between pathogens will be modelled through the use of literature derived risk ratios for each variable (such as teat dipping and dry cow therapy). The combined effect of all modelled risk factors will be to scale the probability of new infection as predicted by the regression of Houben et al. (1993) or the new infection probability distribution data of Gunn et al., (1999) by the risk ratio. The effectiveness of this approach will not be determined until a working model has been built and test simulations have been conducted.

Once a working model has been constructed, verification and validation of the model must occur before final release. This will occur through engagement of domain experts to assess model output.

Acknowledgments

I wish to thank the Dairy Research and Development Corporation for funding this work. Special thanks for guidance and advice go to Dr Pauline Brightling, Professor Graeme Mein and Dr Michael Larcombe. These three people provide a wealth of knowledge on mastitis, milking machines, the Australian dairy industry and simulation modelling. I look forward to their future contributions.

General Discussion

Mastitis is a major disease of dairy cattle. Financial losses due to mastitis are substantial and arise in a number of ways including reduced milk yield, reduced milk quality, and increased culling rates, treatment costs and prevention costs. Studies have indicated that mastitis is the most expensive disease on dairy farms in terms of disease cost and expenditure on prevention.

The current world trend towards the elimination of domestic tariffs has had major implications on trade. Reduced protectionism has resulted in increased use of non-tariff trade barriers to protect markets with quality standards of supplied product assuming greater importance. Mastitis, and especially subclinical mastitis, is a major cause of reduced product quality in the dairy industry. This effect is predominantly mediated through increased somatic cell counts (SCC) in milk. Failure to control mastitis can be expected to result in reduced prices for dairy products and loss of export markets. The economic effects of mastitis on major dairy product exporting countries like Australia and New Zealand is likely to increase in the future. It has become imperative for Australia and New Zealand to become world leaders in dairy product quality.

The increasing importance of product quality implies that the current level of mastitis present within the national herd can no longer be tolerated. The Dairy Research and Development Corporation of Australia has increased funding for mastitis research and extension during the last five years. The national mastitis control and extension program, Countdown Downunder, has defined two national goals for the industry to achieve by the year 2001:

1. A minimum of 90% of Australian dairy farms supplying milk to have BMSCC's less than 250,000 cells/ml in all milk supply periods

2. All Australian dairy farms supplying milk to have BMSCC's less than 400,000 cells/ml in all milk supply periods.

The key to the success of Countdown Downunder in meeting these goals will depend upon effective transfer of knowledge to dairy farmers, industry advisors, milk factories and industry stakeholders. The development of recommendations specific to the Australian dairy industry is vital for success.

The dairy industry in Australia and New Zealand is unique in that it is comprised of predominantly pasture based family farms with seasonally calving herds. Both countries have export driven dairy industries. Producers are highly exposed to the low world dairy commodity prices and this has placed increased financial pressure on producers. The average Australian herd size is increasing beyond 180 cows as producers seek economies of scale, however the real returns from dairy farming are reducing. These pressures make the cost-benefits of individual mastitis control points much more marginal for producers in Australia and New Zealand compared to many other countries with greater domestic price support. The large herd size and the seasonality of calving in many herds have also shifted the dynamics of infection within the industry over time; for example an increase in incidence of environmental mastitis due to pathogens such as *S. uberus* is being observed. The combination of low commodity prices, low real returns for dairy farmers, large herd sizes and seasonal calving has meant that the a national mastitis control program must be tailored for the Australian industry.

Many mastitis control points currently used within the Australian industry have been insufficiently studied or evaluated. A key initial step in the development of Countdown Downunder was an extensive review of the international literature on the epidemiology and economics of mastitis control. Emphasis was placed upon studies that could be applied to the Australian industry. The author was one member of a team of industry analysts who assisted with the review. Part of this review formed chapter one of this dissertation.

The major pathogen of the Australian dairy industry over the next decade is likely to be *S. uberis*. Over half of all culture results from cows with mastitis at commercial laboratories within the seasonal calving areas include *S. uberis* (J. Browning pers. comm.). *S. uberis* is an environmental pathogen that is most commonly associated with new infections at calving, in large herds with high stocking rates and during periods of heavy rain. The epidemiology of infection with *S. uberis* has not been fully elucidated. Some studies have indicated that non encapsulated strains of *S. uberis* display better invasive abilities than encapsulated forms (Almeida et al., 1996 b). At present there is little published evidence of cow-to-cow spread

of *S. uberis*, (Lam et al., 1996) however there is evidence supporting a correlation between teat end damage (loss of keratin and hypoxia) and incidence of infection with *S. uberis*. The role of teat end damage from the milking machines in new infections with *S. uberis* requires investigation. As herds become larger, increased exposure to *S. uberis* is likely. Overmilking is also increasing in large herds and this is resulting in increased teat end damage. Dry cow therapy appears to offer a protective role against infection with *S. uberis* in the late dry / early lactation period (Natzke et al., 1974; Mein at al., 1990; Osteras et al., 1994). Blanket dry cow therapy recommendations for Australian herds are a likely outcome of these studies.

The most common cause of high SCC in Australian dairy cows is still *S. aureus*, however the prevalence of this pathogen is decreasing as simple infection controls at milking improve across the industry. Most Australian farmers are financially unable to cull chronically *S. aureus* infected cows based solely upon infections status. There is strong industry demand for an economical treatment of cows with subclinical mastitis during lactation that will produce a significant improvement in milk quality for the remainder of the lactation. A clinical trial investigating the effectiveness of treatment of cows with high SCC during lactation was undertaken to determine the role of therapy in improving milk quality for the remainder of the lactation. Treatment during lactation with intramammary and parenteral antibiotics was found to be ineffective. *S. aureus* infected cows did not demonstrate a significant reduction in the level of infection, SCC or cow survival following treatment when compared to untreated controls. Emphasis upon control of spread of new infection at milking and methods for increasing heifer replacement rates will continue to be the key control points for *S. aurues.*

Obligate udder pathogens such as *S. agalactiae* are becoming less common in endemic situations as awareness of basic contagious pathogen control techniques improve. Whole herd *S. agalactiae* outbreaks are becoming the more common form of the disease within Australia. The presence of outbreaks indicates that high levels of awareness of the basic control points for contagious mastitis pathogens must be maintained. Milking machine examinations by qualified technicians every six months and the use of a recommended teat disinfectant on all teats after every milking will be emphasised. Effective treatment of clinical cows during lactation, use of dry cow therapy on every cow at drying off and the culling of cows with repeat episodes of clinical mastitis during lactation remain current recommendations of Countdown Downunder.

Significant water quality factors influencing the efficacy of teat disinfection have been identified. In Australia, teat disinfection is mixed daily with local water prior to use. The quality of the water used will influence ability of the teat disinfectant to kill bacteria. Iodine based teat disinfectants lose effectiveness when mixed with excessively alkaline water. Chlorhexidine teat disinfectants have reduced killing power if mixed with hard water or water with high levels of iron. Water with high levels of organic matter or suspended solids will reduce the efficacy of all teat dips. Many areas of Australia use bores or irrigation channels for water. Local research with follow up extension into effective sources of water for teat disinfectant has been identified as a priority of Countdown Downunder. Currently, the general recommendation of Countdown Downunder is only to mix teat dip with water that you would be happy to drink.

Many of the control points present within Countdown Downunder have not been quantified in terms of their effect upon either the incidence of mastitis within herds or the economics of implementation of the control recommendations. Problems of this nature within Countdown Downunder have prompted the development of a stochastic simulation model of mastitis within Australian dairy herds. A model for *S. uberis* and *S. aureus* was commissioned. Production of a working model may allow identification of the critical control points for each modelled pathogen, the current deficiencies of knowledge, and the likely economic impact of implementation of a control program on a regional basis.

Australian studies were used to identify the effect of infection with *S. uberis* and *S. aureus* upon production and somatic cell count in individual cows. The mixed regressions obtained will be used to estimate the economic effect of infection of cows with an individual pathogen within a herd. International literature review indicated that there are major deficiencies in knowledge concerning transition probabilities for individual mastitis risk factors. Where possible risk ratios have been calculated and these will been used for model construction.

It is likely that the final model will require modification of initial risk ratios for many variables. If the model cannot be made to mirror Australian field observations on mastitis, specific studies to elucidate the effects of these poorly defined risk factors will be required. Identification of areas requiring further research is one of the objectives of the model building exercise. When a working and validated final model is available it will be used to tailor delivery of Countdown Downunder on a regional basis within Australia.

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