Longitudinal Studies of Contagious Mastitis in a High Somatic Cell Count Dairy Herd

by

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Abstract

The aim of this study was to investigate a sixty cow dairy herd with a persistently high bulk milk somatic cell count (BMSCC) and to find ways to control the problem.

Investigations were initiated in November 1996 following notification to the farmer by the milk purchaser of possible termination of the milk contract if the BMSCC were not reduced to an acceptable level. The BMSCC had been greater than the EC standard of 400,000 cells/ml of milk since the winter of 1995. Investigation of the high BMSCC began by assessing the milking routine and by the measuring individual cow somatic cell counts (ICSCC). Cows were subsequently selected for California Mastitis Testing (CMT) on the basis of ICSCC > 400,000 cells/ml. Individual quarter samples, positive on CMT, were submitted for bacteriological culture. The results showed 67% of cows sampled to have infection with *Streptococcus agalactiae* and 43% to have infection with *Staphylococcus aureus*. The milking routine and general management on the farm were poor. Improvements in record keeping, general management and strict adherence to the mastitis control programme termed the 'Five Point Plan' were advised. Following uptake of these recommendations, whole herd 'blitz therapy' was implemented. The aim of the chosen treatment was to eliminate *Streptococcus agalactiae* infection from the herd and to produce a rapid reduction in the BMSCC, thereby allowing the farmer to continue to market the milk. The 'blitz therapy' comprised of intramammary treatment, with nafcillin, dihydrostreptomycin and benzyl penicillin (Nafpenzal MC; Intervet UK Limited), and parenteral treatment with penicillin (Depocillin; Intervet UK Limited), of all lactating cows; and parenteral treatment with amoxycillin and clavulanic acid (Synulox Ready-To-Use Injection; Pfizer Limited), of the dry cows and maiden heifers. The milk was discarded for the longest recommended withdrawal period for the drugs used and the milk purchaser tested the milk for antibiotic residues prior to collection for commercial sale.

In order to assess the response to the management changes and to the whole herd 'blitz therapy', weekly BMSCC were monitored and bulk milk samples were collected for bacteriological culture weekly for 12 weeks, and then monthly for the next 9 months. In addition, bacteriological culture of individual quarter samples from all the lactating cows in the herd was carried out on two occasions following the 'blitz therapy', and from selected cows on two later occasions and from cows which calved within one month of the 'blitz therapy'. Individual quarter somatic cell counts (IQSCC) were carried out on the cows sampled during the herd screenings and were mostly within acceptable limits, i.e. < 250,000 cells/ml. The BMSCC was reduced to below 400,000 cells/ml for at least one year after the 'blitz therapy'. *Streptococcus agalactiae* was not isolated from any of the bulk milk samples or from any of the cows, with the exception of one cow which had been dry at the time of the herd treatment. This cow was isolated immediately and treated. Repeated sampling from this cow failed to detect *Streptococcus agalactiae* infection and to date, the herd has remained free of *Streptococcus agalactiae* infection for one year.

The isolation of *Staphylococcus aureus* from 10/22 weekly or monthly bulk tank samples and also from 28% of the cows on one, or more, occasions during the herd screenings led to an attempt to reduce *Staphylococcus aureus* infection in the herd. Six cows from which *Staphylococcus aureus* was isolated from one or more milk samples taken during the herd screenings, were selected for antibiotic treatment. Ceftiofur (Excenel Sterile Powder; Pharmacia and Upjohn Limited) was administered parenterally to these cows once a day for 10 days. Following treatment, monitoring showed that despite the fact that fewer colonies of *Staphylococcus aureus* were cultured the bacteria were still present in the milk of these cows, indicating that this treatment failed to eradicate *Staphylococcus aureus*.

In conclusion, despite failing to eliminate *Staphylococcus aureus* from treated cows, the combined effects of improved management and 'blitz therapy' were successful in bringing about a dramatic reduction in BMSCC, eliminating *Streptococcus agalactiae* and ensuring the farmer was able to market the milk. The reduction in the level of mastitis in this herd has meant greater financial returns for the farmer through increased yield and reduced penalties, in addition to a great improvement in cow welfare on this farm.

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Author's declaration

Other than the help acknowledged, all the work presented in this thesis was carried out by the author. It has not been submitted, in full or in part, for consideration for another degree or professional qualification.

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List of abbreviations

A.I.	Artificial Insemination
A. pyogenes	*Actinomyces pyogenes*
BMSCC	Bulk Milk Somatic Cell Count
BSE	Bovine Spongiform Encephalopathy
CMT	California Mastitis Test
DCT	Dry Cow Therapy
EC	European Community
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
ICSCC	Individual Cow Somatic Cell Count
IDF	International Dairy Federation
IQSCC	Individual Quarter Somatic Cell Count
ppl	pence per litre
REFP	Restriction Enzyme Fragmentation Pattern
SCC	Somatic Cell Count
S. agalactiae	*Streptococcus agalactiae*
S. aureus	*Staphylococcus aureus*
S. dysgalactiae	*Streptococcus dysgalactiae*
S. epidermidis	*Staphylococcus epidermidis*
spp.	species
S. uberis	*Streptococcus uberis*
TBC	Total Bacterial Count

Chapter 1

Introduction

1.1. Mastitis

1.1.1. The disease

Mastitis is defined as inflammation of the mammary gland and is characterised by physical and chemical changes in the milk, expressed, among other things, by an increased number of somatic cells (Tolle, 1975). Milk somatic cells consist of white blood cells (O'Rourke and Blowey, 1992; Hillerton, 1997) and epithelial cells (Hillerton, 1997). Somatic cell counts (SCC) provide a good estimate of the degree of inflammation present in the udder at the time of sampling (Sears and others, 1993; Logue, 1997). White blood cells enter the milk from the blood in response to inflammation and epithelial cells are shed from the lining of the udder tissue (Philpot, 1984; Nickerson, 1985; Blowey and Edmondson, 1995). The entry of mastitis pathogens into the udder induces a massive influx of white blood cells in an attempt to eliminate the bacteria (Sandholm and others, 1990). If the infection is successfully eliminated, the cell count returns to normal, but if the white cell response is unsuccessful, subclinical infection becomes established and there will be a continued secretion of white blood cells into the milk resulting in an increased SCC (Blowey and Edmondson, 1995). Milk from normal, uninfected glands usually has a SCC which is less than 100,000 cells/ml, whereas the average SCC for glands with subclinical infection can increase to 500,000-1 million cells/ml, and for clinical *Staphyloccus aureus* infections the SCC can be approximately 4 million cells/ml and for clinical *Sterptoccus uberis* or coliform infections the SCC can be approximately 20 million cells/ml (Hillerton, 1997).

All herds are susceptible to mastitis (Wilson and Kingwill, 1975). Mastitis is caused by pathogenic micro-organisms, usually bacteria (Wilson and Richards, 1980; Sandholm and others, 1990; Watts, 1990; Sears and others, 1993; Cullor and Tyler,

1996), although the organism may not be present at the time of examination (Sears and others, 1993). Mastitis is a complex disease syndrome, and for convenience is often classified as either contagious mastitis or environmental mastitis (Rebhun, 1995). Contagious mastitis is caused by organisms that can colonise the mammary gland and can be spread by the milking procedure, contaminated machinery (Tolle, 1975; Cullor and Tyler, 1996; Logan and Gillespie, 1996), the hands of milkers (Tolle, 1975; Fox and Gay, 1993; Rebhun, 1995; Cullor and Tyler, 1996; Smith and Hogan, 1996) and nursing calves (Cullor and Tyler, 1996). Environmental mastitis is caused by environmental pathogens that do not normally infect the mammary gland, but can do so when contamination of the cow's environment, teats, udder or milking machine occurs and the organisms gain access to the teat cistern (Rebhun, 1995). A dramatic reduction in the prevalence of contagious pathogens and an associated reduction in bulk milk somatic cell counts (BMSCC) has been seen over the last 30 years (Booth, 1993; Smith and Hogan, 1996). Booth (1985) showed that over a 13 year period, in herds in the UK, a 32% reduction in the average BMSCC was seen, a reduction from 573,000 cells/ml to 310,000 cells/ml.

Mastitis may also be classified as subclinical, where the mammary gland and milk appear normal, or clinical, which is characterised by inflammatory changes in the mammary gland and alteration in the appearance of the milk (Philpot, 1984).

1.1.2. Clinical mastitis

Clinical mastitis can be further classified as peracute, acute, subacute or chronic (Tolle, 1975; Radostits and others, 1994a; Cullor and Tyler, 1996), and is characterised by grossly abnormal milk with varying degrees of mammary gland inflammation (Sears and others, 1993; Cullor and Tyler, 1996). Signs of inflammation include redness, heat, swelling and pain (Blowey, 1990; Cullor and Tyler, 1996) and loss of function of the mammary gland (O'Rourke and Blowey, 1992). When there is severe inflammation with swelling, heat and pain of the quarter and there is a marked systemic reaction mastitis is classified as peracute; when there is severe inflammation but little or no systemic reaction it is classified as acute; when there is mild inflammation with persistently abnormal secretion of the udder it is

classified as subacute; and when there is recurrent bouts of inflammation with little change in the milk it is classified as chronic (Jain, 1979; Radostits and others, 1994a). In peracute mastitis, the clinical signs can be varied, but are usually severe with a sudden onset. The clinical signs can include depression, anorexia, pyrexia and recumbency (Bramley, 1992; Cullor and Tyler, 1996). The systemic signs are often detected before the local inflammation is noticed (Bramley, 1992).

Over a 12 month period Blowey (1984) found the mean incidence of clinical mastitis to be 26.5% of cattle in UK dairy herds: 51 cases occurred per 100 cows suggesting that cows getting mastitis were likely to get clinical mastitis twice in 12 months. Similar rates of mastitis infection were shown in data provided by Kossaibati and Esslemont (1995) in the University of Reading Daisy Report, who found a mean of 33.2 cases of clinical mastitis per 100 cows with 21% of the herd affected with 1.6 cases per affected cow per year.

1.1.3. Subclinical mastitis

Subclinical mastitis occurs when the mammary gland is infected and the number of leucocytes (somatic cells) is increased, but the milk appears to be grossly normal and there is no clinically detectable sign of inflammation in the mammary gland (Sears and others, 1993; Cullor and Tyler, 1996). Mastitis is most often subclinical (Radostits and others, 1994a) and indeed subclinical mastitis was reported by Newbould (1984) to account for 70% of all infected quarters. According to Biggs (1996), an individual cow somatic cell count (ICSCC) of $> 200{,}000$ cells/ml can be taken to indicate subclinical infection. Over time, repeated or persistent bouts of subclinical mastitis will lead to fibrosis of mammary tissue producing firmer glands and a reduction in milk production (Cullor and Tyler, 1996). Contagious pathogens are more likely to produce subclinical infections, and hence high SCC, than environmental pathogens (Eberhart and others, 1982; Blowey and Edmondson, 1995). This is due to the rapid elimination of environmental organisms in cases of environmental mastitis, when high SCC usually occur only during the period of clinical mastitis (Blowey and Edmondson, 1995). However, it should be remembered that chronically infected glands can have low SCC because of the cyclic

shedding of organisms (Sears and others, 1993; Lawrence, 1997). The main cause of high BMSCC in Scottish dairy herds was found to be subclinical infection with *Streptococcus agalactiae* infection, which was found in 83% of the herds sampled, this was assessed from individual cow or quarter sampling (Logue, 1997). *Staphylococcus aureus* infection was found to be the most prevalent pathogen in herds where the BMSCC remained above 400,000 cells/ml for at least one year after the first sampling (Logue, 1997). The average reduction in milk output per quarter per lactation resulting from mastitis has been reported to be 15-40% depending on the type, severity and duration of infection (Hale and others, 1956; Beck and others, 1992).

1.2. Economics

Mastitis is a major disease problem for the dairy industry (Booth, 1988; Sischo and others, 1990; Bramley, 1992; O'Rourke and Blowey, 1992; Smith and Hogan, 1993; Muldoon, 1995), causing significant economic losses (Wilson and Kingwill, 1975; Esslemont and Peeler, 1993; Rebhun, 1995). Mastitis is reported to be the most costly disease of dairy cattle (Roberts and others, 1969; Schmidt, 1969; Watts, 1990; De Graves and Fetrow, 1993). It is quoted as being the most common disease of dairy cattle contributing to culling and is responsible for 10% of premature disposals (Esslemont and Spincer, 1993). Economic losses associated with mastitis are due to loss of production, the cost of treatment and prevention (De Graves and Fetrow, 1993; Reneau, 1993; Radostits and others, 1994b), discarded milk, early culling, drug costs, veterinary expenses, increased labour (De Graves and Fetrow, 1993; Reneau, 1993) and reduced milk yields (Janzen, 1970; Nickerson, 1985).

The greatest loss caused by clinical mastitis is the loss in milk production, this involves the reduction in milk yield and the discarding of milk due to the use of antibiotics (Kossaibati and Esslemont, 1995). Subclinical infections will also decrease the milk yield and alter the composition of the milk (Bramley, 1992; Harmon, 1994). Herds with high BMSCC give lower milk yields than herds with lower BMSCC (Bramley and Dodd, 1984). The lactation milk yield of the average

cow is reduced by 77 kg (77 litres) for every increase of 100,000 somatic cells/ml in the annual mean BMSCC of the herd (Booth, 1981). Winkler (1982) reports a 3% reduction in lactation milk yield with BMSCC 150,000 - 225,000 cells/ml, a 5% reduction with BMSCC 260,000-380,000 cells/ml, 8-12% reduction with BMSCC 420,000-1.2 million cells/ml and 16-20 % reduction in milk production with BMSCC 1.28-2.28 million cells/ml. Studies in Canada demonstrated that milk yields can drop by 2.5% for every 100,000 increase in SCC above the baseline of 200,000 cells/ml (Philpot, 1984). Seventy five percent of losses from subclinical mastitis are accounted for by reduced production (Fetrow, 1980; Philpot, 1984). Unfortunately, 75% of the subclinical losses are 'invisible' to the dairy manager, whereas the costs of treatment, control and prevention are highly 'visible' (Fetrow, 1980). Booth (1981) calculated, from 1980 milk prices, that an annual loss of approximately £87 million was due to reduced milk sales alone i.e. £27 for every dairy cow in the UK at that time. Kossaibati and Esslemont (1995) recorded the average direct cost of a case of mastitis as £119. The direct costs involved in cases of mastitis can vary from £80 for a mild case, £208 for a severe case and £200 for a fatal case: including the replacement of the cow the cost can be as much as £2,214.

1.3. Legislation

Consumer pressure and demands by international markets for high quality dairy products drive milk producers to strive for further improvements in mastitis control (Reneau, 1993; Smith and Hogan, 1996). The European Community Health and Hygiene Directive 92/46, detailing standards for the hygienic quality of milk intended for human consumption, has had major implications for the dairy industry. The EC directive requires bulk milk to have a total bacteria count (TBC) < 100,000 bacteria/ml and SCC < 400,000 cells/ml. In addition to reducing SCC, pressure exists to reduce the incidence of clinical cases of mastitis, to produce milk free of antibiotics, other chemical residues and potential human pathogens (Smith and Hogan, 1993). In order to avoid exceeding the standard for SCC at some time in the year, the recommended target for the annual mean BMSCC should be < 250,000 cells/ml (Logue and others, 1994).

Together with the market pressure, the economics of milk production are responsible for the current interest in reducing mastitis in dairy herds. The EC directive for lower SCC has had a major impact on the UK dairy industry: in March 1991 one fifth of UK producers would have failed to meet the SCC standards. The TBC standard had far less impact, in March 1991 fewer than one producer in a thousand would have failed to meet the standard and even then the failure was only temporary (Logue and others, 1994; Logue, 1997). Now, in 1998, all farmers must meet the standards or face being unable to sell their milk.

1.4. Epidemiology

1.4.1. General

Susceptibility to intramammary infection, and the rate and duration of infection varies widely among dairy herds, and among cows within herds (Philpot, 1975). Mastitis occurs more commonly in older cows and in the early lactation period (Jackson and Bramley, 1983; Bramley, 1992: Radostits and others, 1994a). The higher incidence of mastitis around calving is due to the rate of new infections gained during the dry period, and is thought to be due to suppression of the host defences at that time (Bramley, 1992). The increasing ease of penetration of the teat duct by pathogens is thought to explain the higher incidence of mastitis with increasing age (Bramley, 1992). Genetic factors such as milk yield, quarter position, length of teats and the milkablity or milking speed of the cow also have an affect on the susceptibility to mastitis (Stovlbaek-Pederson, 1975; Tolle, 1975: Radostits and others, 1994a). Teat lesions, particularly at the apex, contribute to an increased incidence of intramammary infection (Philpot, 1975; Sandholm and others, 1990; Radostits and others, 1994a). Tolle (1975) found that, in the absence of teat dipping, all teat lesions became secondarily infected and acted as reservoirs for mastitis pathogens. Environmental factors which reduce the udder's resistance to infection, increase the transmission of bacteria from the surroundings and from infected cows to healthy cows, and increase the possibility of bacteria penetrating the teat canal, will lead to increased incidence of mastitis (Stovlbaek-Pederson, 1975).

1.4.2. Somatic cell counting

Somatic cell counting is a practical and effective method to evaluate a herds mastitis status and gives a good indication of the amount of infection in a herd (Booth, 1985; Dohoo and Meek, 1982; Sears and others, 1993; Cullor and Tyler, 1996). Intramammary infection is the most important factor influencing the number of somatic cells in milk (Reneau, 1986). Other factors which influence the somatic cell count include breed, age, stage of lactation and physiological stresses such as excitement and high temperatures and trauma (Reichmuth, 1975; Tolle, 1975; Philpot, 1984; Rebhun, 1995). The variation in SCC observed among different breeds may reflect the differing management systems they typically experience (Reichmuth, 1975; Radostits and others, 1994a). The SCC tends to increase with the age of dairy cows, which may be related to the increased period of exposure to potential pathogens, teat canal damage and poorer immune responses (Reichmuth, 1975; Blowey and Edmondson, 1995). However, in cows older than seven years of age, the average SCC is reduced, but this is probably due to the retention of cows with low cell counts, whereas high cell count cows are often selected for early culling (Reichmuth, 1975).

There is a rise in somatic cell count between the first and third days of lactation (Cullen, 1968; Reichmuth, 1975; Philpot, 1984; Blowey and Edmondson, 1995; Rebhun, 1995), and throughout lactation there tends to be a rise in SCC due to the presence of infection (Reichmuth, 1975). There is also a regular cyclical rise and fall in SCC during lactation which is probably due to desquamation of epithelial cells or increased alveolar permeability allowing more cellular exudate to be released (Cullen, 1968). The increase in SCC towards the end of lactation is exaggerated by the reduction in the volume of milk secreted as lactation advances (Reichmuth, 1975; Blowey and Edmondson, 1995; Rebhun, 1995). Stressors can exacerbate the inflammatory response which is already present in an irritated udder (Reichmuth, 1975). Stress related increases in SCC may be caused by errors in feeding methods, oestrus and irregular milking intervals (Reichmuth, 1975). Short milking intervals tend to be associated with higher SCC because longer intervals allow more secretion

to be produced thereby diluting the number of somatic cells in the milk: the so-called dilution effect (O'Rourke and Blowey, 1992). BMSCC is regarded as a measure of the prevalence of infection in a herd and has been used as a measure of bulk milk quality such that payment schemes have been devised to penalise high BMSCC herds (Westgarth, 1975; Booth, 1996; Logue, 1997). Financial penalties for high BMSCC act as an incentive for farmers to control mastitis and are a useful means to assess progress of control programmes (Booth, 1981).

Somatic cell counting can be done at the quarter level when individual milk samples are collected from each quarter i.e. individual quarter somatic cell count (IQSCC), or at the cow level, when a composite sample of equal volumes of milk is collected from all quarters of the cow into one container i.e. individual cow somatic cell count (ICSCC), or at the bulk tank level i.e. bulk milk somatic cell count (BMSCC).

1.5. Contagious pathogens

Contagious organisms associated with mastitis include *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae* (Natzke, 1981; Dodd, 1983; Smith, 1983) and *Mycoplasma spp.* (Rebhun, 1995). Subclinical mastitis is most commonly associated with *S. aureus* and *S. agalactiae* infections (Sears and others, 1993; Cullor and Tyler, 1996). *Streptococcus agalactiae* and/or *S. aureus* have been shown to be responsible for 85-95% of bovine mastitis cases (Wilson and Kingwill, 1975; Natzke, 1981; Dodd, 1983; Mercer and Sears, 1983) and have frequently been found to infect more than one quarter of the same cow (Logue, 1997). Gunn (1995) found, in studies of Scottish dairy herds, that *S. agalactiae* and *S. aureus* were the most prevalent mastitis pathogens in high BMSCC herds.

1.5.1. *Staphylococcus aureus*

Staphylococcus aureus is a contagious pathogen which can be found on the skin of the udder and teats as well as in intramammary tissue (McDonald, 1977; Cullor and Tyler, 1996). There are many different strains of *S. aureus* which can cause mastitis

and they have been demonstrated using restriction enzyme fragmentation patterns (Young, 1997). Most *S. aureus* infections are chronic subclinical infections, though occasional clinical cases occur which are mostly mild to moderate in severity (Cullor and Tyler, 1996). These intramammary infections tend to be of a long duration (Bramley and Dodd, 1984). Occasionally, an acute gangrenous toxic mastitis can result from *S. aureus* infection (Bramley, 1992). Acute gangrenous mastitis is recognised initially as a hot, red, swollen gland which rapidly becomes cold and the secretion becomes sero-sanguinous (Radostits and others, 1994a; Cullor and Tyler, 1996). If untreated, the gland becomes discoloured, and this area then sloughs within 10-14 days (Radostits and others, 1994a; Cullor and Tyler, 1996). Affected cows are dehydrated, depressed, anorexic, pyrexic and exhibit signs of toxaemia (Radostits and others, 1994a; Cullor and Tyler, 1996). Fortunately, gangrenous mastitis is not common (Cullor and Tyler, 1996).

In a study of Scottish dairy herds by Logue (1997), *S. aureus* was the most common cause of both subclinical and clinical mastitis from samples sent to veterinary investigation centres. *Staphylococcus aureus* is difficult to eliminate as it can survive for extended periods on the skin (McDonald, 1977) and can survive within neutrophils, which serves to protect it from antibiotics (Craven and Anderson, 1979; Sandholm and others, 1990). Important sources of *S. aureus* infection within the dairy herd are infected mammary glands (Davidson, 1961) and infected teat lesions (Francis, 1980; Bramley, 1992). Intensive intramammary therapy is often unsuccessful in eliminating infection with *S. aureus* and culling may be necessary (McDonald, 1977). Antibiotic treatment of *S. aureus* is reported to result in a poor success rate and is considered to be a major limitation in the effectiveness of mastitis therapy (Bramley and Dodd, 1984). The exception to this rule is clinical staphylococcal infections where the clinical response to therapy is good i.e. reduction in SCC and resolution of clinical signs of mastitis, though less than 35% of the infections are eliminated. The reasons for poor clearance of staphylococcal infections may be due to resultant mammary abscessation, the intracellular position of bacteria within neutrophils (Madgwick and others, 1989) and antibiotic resistance due to β-lactamase production and virulence factors (Sandholm and others, 1990;

Fox and Gay, 1993). β-lactamase is produced by *S. aureus* and renders the β-lactam ring in penicillins inactive (Sandholm and others, 1990; McKellar, 1991; Vestweber and Leipold, 1993).

1.5.2. *Streptococcus agalactiae*

Streptococcus agalactiae is a highly contagious obligate pathogen of the bovine mammary gland (McDonald, 1977; Biggs, 1996; Cullor and Tyler, 1996). Logue (1997) reported that *S. agalactiae* was the second most common isolate in herds tested, but was not commonly a cause of clinical cases of mastitis. The primary site of colonisation in infection is udder tissue, but teat lesions and teat ducts can frequently be colonised (Dodd, 1983). *Streptococcus agalactiae* dies when exposed on healthy or eroded skin, is sensitive to penicillin (Plommet and LeLouedee, 1975), and can be eradicated from herds (Philpot, 1975; McDonald, 1977). Infection is almost always introduced into a herd by purchased cattle (Roberts and others, 1969; Biggs, 1996). In *S. agalactiae* infection, there is often only one quarter infected, but it is possible for the other quarters to become infected, especially in herds where the infection is endemic (Biggs, 1996). Infected cows have increased somatic cell counts at all stages of subclinical infection (Blowey and Edmondson, 1995) and the milk yield can be reduced by approximately 10% (Hale and others, 1956). However, both the ICSCC and the excretion of bacteria in milk can vary considerably (Blowey and Edmondson, 1995). If the BMSCC has been increased for a number of years in a herd where *S. agalactiae* has been isolated, then the infection is likely to be endemic (Biggs, 1996). In this situation, ICSCC for some cows in the herd could be in the region of 1,000,000-2,000,000 cells/ml could be expected (Biggs, 1996). In herds where *S. agalactiae* infection is responsible for BMSCC > 500,000 cells/ml, organisms can be readily isolated from the bulk milk samples (Blowey and Edmondson, 1995). *Streptococcus agalactiae* is the only mastitis pathogen likely to respond well to treatment during lactation (Philpot, 1984). The incidence of *S. agalactiae* infection is reduced significantly by adopting good milking hygiene practices and good milking technique (Philpot, 1975).

1.6. Diagnosis

Diagnostic tests are used to identify the nature and extent of a mastitis problem in a dairy herd or to identify an individual animal for segregation, treatment or culling (Bramley, 1992). Somatic cell counting is used to diagnose subclinical infections at the quarter, cow or herd level (Newbould, 1984; Cullor and Tyler, 1996).

1.6.1. Milk sample collection

In order to diagnose mastitis pathogens accurately, milk samples need to be taken carefully (Watts, 1990). Poor sampling technique often leads to contamination of milk samples and confusion over the significance of the organisms isolated (Buswell, 1995).

1.6.2. Diagnostic tests

1.6.2.1. California Mastitis Test

The California Mastitis Test (CMT) is an indirect method of estimating SCC and is based on a gelling reaction between the nucleic acid of the somatic cells and an anionic surface active agent with bromcresol-violet as an indicator (Scalm and Noorlander, 1957; Philpot, 1984; Bramley, 1992; Radostits and others, 1994b; Muldoon, 1995). Although not very sensitive or specific, the CMT is a practical and useful on-farm test to identify individual cows with subclinical mastitis (Jaartsveld, 1975). This test is not sensitive enough for bulk milk samples: the results are usually negative or a small amount of gel formation occurs, so that automated SCC should be used for assessment of bulk milk (Cullor and Tyler, 1996).

1.6.2.2. Bacteriology and somatic cell counting

Bulk milk sampling is a practical means of monitoring a herd for potential mastitis pathogens, but obviously is far less sensitive than culturing individual quarters (Lawrence, 1997). Bulk milk bacteriological examinations have a high specificity but low sensitivity as a test for mastitis, meaning that some infected herds may not be detected on bulk milk samples (Godkin and Leslie, 1991). Biggs (1996) recommends that bacteriology is performed on bulk milk and, as necessary, from

selected high ICSCC cows. An infected quarter is defined as one producing milk from which bacteria are recovered in pure culture and in which there is an increased number of leucocytes, compared to a normal quarter of the same cow (Newbould, 1984). Sears (1993) suggests that ICSCC > 300,000 cells/ml should be used as a guideline for sampling for bacteriological culture. Sampling high ICSCC cows increases the efficiency of bacteriological cultures (Erskine and Eberhart, 1990; Logue, 1997). Cows infected subclinically may be cyclic shedders of organisms and may vary between low and high shedding patterns throughout a lactation (Sears and others, 1990; Sears and others, 1993; Shukken and others, 1993). The daily variation in shedding of bacteria, in certain cows, has been reported to fluctuate by a hundred fold (Lawrence, 1997). The accepted criterion for diagnosis of intramammary infection is that intramammary infection exists when the same organism is isolated from two, or two out of three, consecutive samples taken at least one day apart (Sears and others, 1993). Twenty five percent of *S. aureus* infected cows will have a negative single culture result i.e. false negative, but with repeated sampling sensitivity is increased to 94-98% (Sears and others, 1993). Observations in Minnesota have shown that, in order to eliminate false negatives attributable to the daily variation in shedding of *S. aureus,* milk samples need to be taken for four consecutive days (Farnsworth, 1993). However, Erskine and Eberhart (1988) compared duplicate and single samples taken for mastitis pathogen identification and found a high level of agreement for the contagious pathogens, but a high level of disagreement for the environmental pathogens. Duplicate samples should be taken within one week of each other to reduce the chance of missing new infections (Neave, 1975). Therefore, a single sample culture is acceptable for the identification of contagious pathogens, such as *S. aureus* and *S. agalactiae*, allowing savings on labour and laboratory costs (Erskine and Eberhart, 1988). However, neither single nor duplicate samples offer a high degree of accuracy in identification of intramammary infection with environmental pathogens. This is thought be due to the low numbers of environmental pathogens in the milk (Neave, 1975) and the fact that contamination is likely to be due to the environmental reservoir of infection (Smith, 1983). In order to improve the accuracy of diagnosis in environmental mastitis, repeated cultures with larger volumes of milk (> 0.01 ml) and CMT should be used

in conjunction with a consideration of clinical signs, appearance of milk and ICSCC (Erskine and Eberhart, 1988).

Bacteriological examination of bulk milk and, if necessary, milk from high ICSCC cows, should be carried out to check for *S. agalactiae* infection in a herd (Biggs, 1996). Biggs (1996) found that if *S. agalactiae* infection was widespread among cattle in a herd, such that BMSCC was > 500,000 cells/ml, then *S. agalactiae* was usually readily isolated from bulk milk samples. Quarters infected with *S. agalactiae* usually shed high numbers of bacteria, but occasionally so few organisms are shed that a single sample can be negative on culture (Dinsmore and others, 1991). Underlying *S. agalactiae* infection may be masked if a heavy growth of another pathogen is cultured (Biggs, 1996).

1.7. Pathogenesis

1.7.1. General

The primary defence mechanism of the mammary gland is the teat canal, as this represents the portal of entry for nearly all mastitis-causing micro-organisms (Bramley and Dodd, 1984; Craven and Williams, 1985; Nickerson, 1985; Sandholm and others, 1990; Bramley, 1992; Vestweber and Leipold, 1993). Injury to the teat end and teat canal almost always leads to intramammary infection (Benedixen, 1935; Ferguson, 1944; Smith and Hogan, 1996). The development of intramammary infections occurs by the contamination of the teat end with pathogenic organisms (Tolle, 1975; Bramley and Dodd, 1984) followed by penetration into the teat canal and the colonisation by the organisms in the sinuses, ducts or alveolar tissue (Kingwill, 1980; Bramley and Dodd, 1984). Any infection gaining access to the gland must remain adherent to the inner surfaces, despite the out-flow of milk, and escape the action of the defence mechanisms (Reichmuth, 1975). The division of the udder into four independent quarters helps to restrict the spread of infection (Reichmuth, 1975). However, the transfer of some pathogens between quarters is inevitable at milking time, even under the best hygienic conditions (Philpot, 1975).

Most infections which occur in the dry period develop in the first two or three weeks after drying off and are thought to be due to pathogens remaining in the udder from the milking period (Philpot, 1975).

1.7.2. *Staphylococcus aureus*

There is considerable variation in the type of mastitis caused by *S. aureus*, this is not thought to be attributed to differing virulence between strains. Instead, it appears to be related to the infective dose or the lactational status of the cow (Bramley, 1992; Radostits and others, 1994a). Infection early in lactation tends to result in peracute mastitis, whereas infection later on or in the dry period results in acute or chronic infections (Radostits and others, 1994a). Peracute mastitis results from the uncontrolled growth of the organism (Bramley, 1992) and leads to thrombosis of the mammary veins which produces local oedema and congestion of the udder. Toxaemia arises from the release of bacterial toxins and tissue destruction (Radostits and others, 1994a). Acute and chronic mastitis differ depending on the degree of involvement of the mammary tissue. The proliferation of bacteria in the collecting ducts occurs in both acute and chronic mastitis, but in acute mastitis fibrin clots block the ducts causing severe reaction in the obstructed area (Radostits and others, 1994a). In contrast, in chronic infections reactions are restricted to the epithelium of the ducts (Radostits and others, 1994a). Abscesses, granulomas and fibrosis develop in some *S. aureus* infections (Bramley, 1992; Radostits and others, 1994a). *Staphylococcus aureus* can survive within polymorphonuclear leucocytes, macrophages and epithelial cells and is thus protected to some extent from some antibiotics (Sandholm and others, 1990; Bramley, 1992).

1.7.3. *Streptococcus agalactiae*

Streptococcus agalactiae does not enter the glandular tissue but is restricted to the epithelial surfaces (Sandholm and others, 1990; Radostits and others, 1994b) and, therefore, is more accessible to antibiotics and the host's own immune mechanisms.

1.8. Treatment

1.8.1. General

In order to treat mastitis successfully, a drug must maintain an effective dose, i.e. exceed the minimum inhibitory concentration (MIC), for an adequate period of time (Bramley and Dodd, 1984; Ziv, 1992). Furthermore, to achieve maximum penetration, antibiotics should be lipid soluble, non- or weakly ionised in milk, and not readily bound by serum or milk proteins (Bramley and Dodd, 1984; Sandholm and others, 1990). Many antibiotics are less effective in milk than suggested by antibiotic sensitivity plates (Sandholm and others, 1990; Blowey and Edmondson, 1995). A choice can be made between bactericidal antibiotics which kill bacteria, and bacteriostatic antibiotics which prevent bacterial growth and multiplication and rely on the cows own defence mechanisms to overcome the infection (Blowey and Edmondson, 1995). Although ostensibly more attractive, bactericidal antibiotics may cause sudden bacterial death and release of endotoxins leading to further problems (Blowey and Edmondson, 1995). The cost of the treatment and the length of milk withdrawal periods should be considered when choosing an antibiotic for mastitis therapy (Blowey and Edmondson, 1995). It is difficult to find an antibiotic which will satisfy all the desired criteria, and some that do so may be either too expensive or reserved for human use (Bramley and Dodd, 1984). With intramammary treatment, the majority of the antibiotic tends to remain within the treated quarter, but some will diffuse into the blood, circulate around the body and be deposited into untreated quarters: therefore the milk from all quarters should be withheld from the bulk tank for the prescribed withdrawal period for that particular preparation (Blowey and Edmondson, 1995). However, the withholding of milk from all quarters of a cow which has been treated with an intramammary preparation is a legal requirement anyway. Treatment efficacies for intramammary infections during lactation reported by Smith and Hogan (1996) were: 80-90% for *S. agalactiae*, 50-60% for coagulase negative staphylococcal infections, 50% for environmental streptococcal infections, 30-50% for *S. aureus*, < 10% for gram negative infections and no response was seen in infections caused by yeasts, moulds, *Mycoplasma* spp. and *Protothea* spp.. Similar cure rates were recorded by Huber (1977): 100% for *S.*

dysgalactiae, 98% for *S. agalactiae*, 82% for *Streptococcus uberis* and only 35% for *S. aureus*. Therapy is more effective in the dry period than during lactation, and is more effective after the first clinical episode, i.e. in heifers, than after repeated treatments, i.e. older cows (Sandholm and others, 1990; Meany, 1992; Lawrence, 1997). Spontaneous recoveries often equal the number of infections eliminated by antibiotic therapy in lactating cows (Griffin and others, 1982), but spontaneous recoveries often take a long time i.e. there will be a prolonged period of infection before recovery occurs (Bramley and Dodd, 1984). The proportion of spontaneous elimination is low for staphylococcal infections at < 20%, high for *Escherichia coli* at > 70%, and intermediate for streptococcal infections (Bramley, 1992). Dodd and Griffin (1975) reported that 90% of staphylococcal infections that did not receive antibiotic therapy persisted for three months, and that over 80% of staphylococcal infections present at drying-off persisted until calving. Bramley and Dodd (1984) found that in most clinically infected quarters a response is seen within one or two days of antibiotic therapy, and in the majority the infection is eliminated.

Treatment of lactating cows with antibiotics, based on increased SCC or bacteriological culture of milk, is not always considered to be cost-effective (Yamagata and others, 1987). This is mainly due to the fact that milk production does not always improve in response to antibiotic therapy: *Staphylococcus aureus* infections respond poorly to antibiotics and some organisms are cleared from the mammary gland without therapy (McLeod and Wilson, 1951). Two suggested approaches to improve the bacteriological cure in lactating cows are, (1) increasing the duration of treatment with intramammary infusions or, (2) combining intramammary infusions with parenteral antibiotics (Lawrence, 1997). In a study comparing the efficacy of three different treatment regimens, Jarp and others (1989) showed that the duration of the treatment is more important than the route of administration in curing cases of mastitis.

1.8.2. Objectives of mastitis treatment

Treatment of mastitis is carried out for many reasons: (1) to treat clinical infections in order to regain normal milk secretion, prevent progression of the disease and

improve the welfare of the cow, (2) to eliminate clinical or subclinical infections, or at least reduce their number and frequency, so that the milk yield from the herd is improved, (3) to prevent the spread of infection, by eliminating the main source of bacteria, in the case of infectious organisms, (4) to prevent development of infection in an animal or herd exposed to a particular or temporary risk, and/or (5) to avoid penalties imposed by milk purchasers for milk with high SCC (Plommet and LeLouedee, 1975; Blowey and Edmondson, 1995).

1.8.3. Antibiotics

The intramammary route is a practical, inexpensive and convenient route for the treatment of bovine mastitis (McKellar, 1991; Vestweber 1993). However, in acute severe disease, the distribution of intramammary antibiotics may be impaired by inflammation within the udder, and therefore parenteral antibiotics may be necessary (Sandholm and others, 1990; Prescott and Baggot, 1993). The choice of antibiotic will depend on the *in vitro* sensitivity of the organism, and it should be remembered that the sensitivity may change over a period of time, especially following intensive antibiotic usage, and also with geographic location (Owens and Watts, 1988; McKellar, 1991). The antibiotic for mastitis therapy should be chosen after considering the activity and pharmacodynamics of the drug, the probable cause of mastitis, the milk withdrawal period, (McKellar, 1991) and the cost, though the cost of discarded milk is often considered to be more important than the cost of the antibiotic (Prescott and Baggot, 1993).

Streptococcus agalactiae (Chapter 1.5.2) is highly sensitive to penicillin and this would be the drug of choice to treat *S. agalactiae* infections (Sandholm and others, 1990). However, *S. aureus* (Chapter 1.5.1.) infections pose more problems. Dodd and others (1964) reported approximately 70% of *S. aureus* isolates causing mastitis to be resistant to penicillin. Wright (1983) also found 51% of *S. aureus* isolates to be resistent to penicillin. Nafcillin and cloxacillin are modified penicillins which are effective against β-lactamase producing *S. aureus* but, like penicillin, they have no action against gram-negative organisms (McKellar, 1991; McKellar, 1996). There are modified penicillins, ampicillin and amoxycillin, which are effective against

gram-negative and gram-positive organisms, but are inactivated by β-lactamase producing *S. aureus* (McKellar, 1991). Clavulanic acid combined with amoxycillin provides irreversible inhibition of β-lactamase produced by *S. aureus*, therefore producing an antibiotic with activity against the vast majority of mastitis causing organisms (Sandholm and others, 1990; McKellar, 1991; McKellar, 1996). Cephalosporins have activity against gram-negative and gram-positive organisms as well as β-lactamase producing *S. aureus*, however, udder penetration by these drugs is not as good as with penicillins (Blowey and Edmondson, 1995). Macrolides such as erythromycin penetrate the udder well and have good activity against gram-positive organisms, including β-lactamase producing *S. aureus* (Ziv, 1980; Sandholm and others, 1990; McKellar, 1991; Prescott and Baggot, 1993). Aminoglycosides such as streptomycin, dihydrostreptomycin and neomycin are often combined with penicillins as they have good activity against coliforms and β-lactamase producing *S. aureus*, but have poor udder penetration (Barragry, 1994). Tetracyclines are broad spectrum and have good activity against gram-negative and gram-positive organisms, including some action against β-lactamase producing *S. aureus*, but udder penetration is limited and strains of resistant coliforms may develop (Barragry, 1994). Novobiocin is a narrow spectrum antibiotic with excellent action against *S. aureus*, which also has synergistic properties when combined with penicillin (McKellar, 1991; Prescott and Baggot, 1993; McKellar, 1996).

In human medicine, penicillin resistant *S. aureus* infections are also a problem, and treatments of choice would be cloxacillin or flucloxacillin, with possible alternatives being first generation cephalosporins, amoxycillin/clavulanic acid combinations or erythromycin (Turnidge and Grayson, 1993). In a study by Efrat and others (1995) it was found that 85% of cases of mastitis in human neonates were due to *S. aureus* infections, and 50% of infections responded to intravenous cloxacillin or amoxycillin/clavulanic acid combination. Brglez and others (1988) reported *S. agalactiae* infections in humans with the most severe cases occurring in the perinatal period, causing abortions and infections in the neonates: with the mother being the main source of infection.

Owens and others (1988) showed that in the cow the combination of parenteral procaine penicillin with intramammary infusions of amoxycillin was more efficacious than intramammary amoxycillin alone in the treatment of subclinical *S. aureus*. The combination of parenteral and intramammary antibiotics produced a bacteriological cure in 51% of quarters and 48% of cows, whereas intramammary treatment alone gave cure rates of 25% of quarters and 30% of cows. The reduced patency of the duct system due to chronic inflammation may contribute to the poor response to intramammary treatment seen in *S. aureus* infections, as intramammary antibiotics must diffuse through the milk into the terminal alveoli through the ducts (Craven and Anderson, 1979).

Corticosteriods are often added to antibiotics in intramammary preparations to reduce inflammation in the udder tissue. Prednisolone has not been shown to adversely affect the function of white blood cells in the udder, despite being proven to be immunosuppressive in other parts of the body (McKellar, 1991). Experiments by Bywater and others (1988) showed that prednisolone effectively increased the rate of reduction of the swelling seen in mastitis with no effect on SCC in milk.

As non-lactating (dry) mammary glands are believed to be naturally resistant to gram-negative organisms, due to the production of lactoferrins, dry cow therapy (Chapter 1.9.1.) is aimed at eliminating intramammary infections caused by *S. aureus* and *S. uberis*. However, Green and Bradley (1998) have found that new infections of *E. coli* have been acquired in the dry period. *Actinomyces pyogenes* should be targeted if summer mastitis is a problem (McKellar, 1991).

1.9. Control

Smaller profit margins experienced in dairy farming in recent years mean that producers need to be more efficient, and any mastitis control needs to be cost effective (Smith and Hogan, 1996). In order to be effective, a control system should be practical and economic (Wilson and Kingwill, 1975; Radostits and others, 1994b) and needs to be continually monitored (Dodd, 1980). Effective and economical

controls for mastitis should rely on prevention rather than treatment (Wilson and Kingwill, 1975; DeGraves and Fetrow, 1993; Erskine, 1993). Mastitis control relies on reducing the exposure of teat ends to pathogens (Bramley, 1980; Cullor and Tyler, 1996; Smith and Hogan, 1996) and/or increasing the resistance of cows to infection (Cullor and Tyler, 1996; Smith and Hogan, 1996).

1.9.1. 'Five Point Plan'

A comprehensive control programme, correctly applied, reduced the occurrence of mastitis by 50% over one year, and by 75% over two to three years in a number of herds (Philpot, 1984). An effective control programme should include good hygienic practise and good management, as specific control measures are not thought to be a substitute for good husbandry (Jackson, 1980).

Success in reducing the exposure of teat ends to infection has followed the application of the so-called 'Five Point Plan', which was developed to control contagious pathogens in the dairy herd. The 'Five Point Plan' was devised by the National Institute for Research in Dairying (NIRD), Central Veterinary Laboratories (CVL), Ministry of Agriculture, Fisheries and Food (MAFF), British Veterinary Association (BVA), National Farmers Union (NFU), Agricultural Training Board and the Association of British Pharmaceutical Societies in November 1971 to attempt to control mastitis in commercial dairy herds (Booth, 1975a). Originally the control regime consisted of six points:

1. Good stock control.
2. Good milk hygiene, specifically thr use of post-milking teat disinfection.
3. Prompt treatment of clinical cases and the use of dry cow therapy (DCT).
4. Culling chronic or recurrent cases.
5. Monitoring progress of control measures by cell counting.
6. Regular testing and maintenance of the milking machine.

Booth (1975b) later adapted these points to make the 'Five Point Plan', which consists of:

1. Post milking teat dipping,
2. Total dry cow therapy (DCT),
3. Proper maintenance and operation of the milking machine,
4. Prompt therapy of clinical cases when they occur and
5. Culling chronic cases.

If hygienic methods are to be of value in reducing the incidence of new infections, they must prevent, or at least greatly reduce, the transmission of pathogens from one teat to another of the same cow, as well as from cow to cow (Philpot, 1975). The reservoir of contagious pathogens can be reduced by DCT, culling, and, to a lesser degree, treatment of clinical cases during lactation (Smith and Hogan, 1996). A high BMSCC caused by *S. agalactiae* infection have proved to be the most responsive to control, whilst those involving *S. aureus* infection alone have been generally unresponsive to any control measures other than selective culling (Logue, 1997). Surveys by Smith and Hogan (1993) and DeGraves and Fetrow (1996) showed that washing udders, single use paper towels, changing cluster liners, post milking teat dipping and DCT were cost-effective improvements to aid the control of mastitis. Good control of subclinical mastitis can be achieved with post-milking teat dipping and DCT of known infected quarters. A marked reduction in *S. agalactiae* and *S. aureus* infections have been seen using this regime (Natzke, 1981; Dodd, 1983; Smith, 1983; Smith and Hogan, 1996). High SCCs, repeated clinical cases and occasional isolation of *S. aureus* are typically suggestive of poor elimination of infection by lactating or dry cow therapy (Bramley, 1992).

1.Post milking teat disinfection.

Post milking teat disinfection is recommended as an effective means to destroy bacteria left on the teat after milking, preventing teat lesions or assisting the healing of teat lesions, and in the control of teat duct bacterial colonisation (Bramley, 1980; Bramley and Dodd, 1984; Bramley, 1992). Trials performed by Neave and others (1966) showed that post milking teat disinfection combined with the use of

individual paper towels or cloths, reduced the incidence of new infections by 50 %.

2. Dry cow therapy.

The aim of DCT is that it is to be used in all quarters of all cows in a herd at drying off to eliminate existing infections and to prevent intramammary infection during the dry period (Neave and others, 1966; Costa and others, 1996; McKellar, 1996). The response to treatment in the dry period is more complete than during lactation. This may be due to the freedom to use higher doses of antibiotics and/or to use long acting preparations when milk withdrawal periods are generally not a concern (Wilson, 1980; Barragry, 1994). The use of DCT is relatively inexpensive as there is no need to discard milk due to antibiotic contamination (Wilson, 1980). Dry cow therapy is very effective in eliminating *S. agalactiae* infections and 80-90% cure rates can be achieved for *Streptococcus spp.* (Philpot, 1984; Barragry, 1994). *Staphylococcus aureus* infections are also much more effectively controlled using DCT with cure rates of 70-80% compared to 30-40% for infections treated during the lactation period. (Philpot, 1984).

3. Milking machine.

There should be regular inspections of the milking machine, as the milking machine is known to influence the incidence of mastitis (Neave and others, 1966; Sandholm and others, 1990; Bramley, 1992). Kirk (1990) stated that improperly functioning or designed milking equipment, especially in conjunction with poor milking technique and hygiene, could greatly increase the level of mastitis in a dairy herd.

4. Prompt therapy of clinical cases.

Clinical cases should be treated promptly to reduce the risk of chronic infection and to maintain the BMSCC and TBC below the acceptable limits. Milking mastitic cows into the bulk tank can lead to wild fluctuations in TBC (David and Jackson, 1984) and result in the BMSCC exceeding the EC standard of < 400,000 cells/ml.

5. Culling chronic cases.

Culling of all infected cows, especially younger or high producing animals, is not usually cost effective or practical (Bramley and Dodd, 1984; Hoblet and Miller, 1991), but may prove to be advantageous in the long term, depending on the pathogen involved (DeGraves and Fetrow, 1996). Culling is recognised as a valuable method of reducing the spread and, in some instances, eliminating certain infectious diseases (Natzke and Everett, 1975). However, culling should not be based on the results of a single sample, whether it be SCC or bacteriological, due to variations in shedding (Chapter 1.6.2.2.) and the possibility of natural elimination of certain pathogens (Blowey and Edmondson, 1995). A cow with persistently increased SCC, i.e. > 250,000 cells/ml for three consecutive monthly samples, should be considered for culling. When making a decision to cull other factors should also be considered, such as the number of cows in the herd in the same category, the number of times the cow has had mastitis in the current and previous lactations, which organisms are involved, milk yield, reproductive status (Reneau, 1993), general health and the source of replacements (Blowey and Edmondson, 1995). The economics involved in the culling of high SCC cows is complex, as the replacement cost and the reduction in milk yield of a heifer compared to a mature cow need to be considered (Bramley and Dodd, 1984; Hoblet and Miller, 1991; Reneau, 1993). It is possible that a cow with chronically high SCC could live comfortably within the herd and could be cured by DCT, but may often be culled for economic reasons. In this way, the direct cost of mastitis treatment will be avoided and a potential source of infection for the rest herd will be removed (Blowey and Edmondson, 1995). However, segregation of the infected cows, or the use of separate milking units for cows infected with *S. aureus* can reduce the prevalence of infection and reduce the BMSCC, and should not be forgotten as a means of mastitis control (Wilson, 1980).

1.9.2. 'Blitz therapy'

'Blitz therapy' is the programmed, simultaneous treatment of all four quarters of cows with intramammary antibiotics aiming to eliminate *S. agalactiae* infection (Roberts and others, 1969). 'Blitz therapy' can be done on a whole herd basis, where the entire milking herd undergo treatment, or on a partial herd basis, where only

selected animals are treated (Blowey and Edmondson, 1995). With adequate case selection, response to therapy is good and the milk withdrawal periods of the drugs commonly used can be short, meaning that this approach can be an economic proposition in a badly infected herd (Yamagata and others, 1987). 'Blitz therapy' should be based on individual quarter or composite bacteriological milk samples (Erskine and Eberhart 1990), as SCC are not a good indicator of the precise diagnosis, even though high SCC correlates well with *S. agalactiae* infection (Blowey and Edmondson, 1995). Herd screening will also identify which cows are infected with other contagious pathogens, will give an indication of the expected SCC response after treatment and will indicate whether further measures may be necessary (Biggs, 1996). The choice between total or partial herd blitz therapy depends on the prevalence of infection within the herd (Biggs, 1996). Total herd 'blitz therapy' gives faster, more predictable results and is more straightforward (Biggs, 1996). However, in herds with a low prevalence of infection, or if the SCC penalties are minimal, then the longer term, less aggressive approach of the partial 'blitz therapy' may be more appropriate and economical (Biggs, 1996). There are reduced costs with partial 'blitz therapy', i.e. fewer intramammary tubes and less milk to discard than if the whole herd were treated simultaneously. However, the necessary repeated herd screening, and treatment of infected cows, as infection spreads during the delay between sampling and treatment may make partial blitz therapy more costly than total herd treatment (Biggs, 1996). A single intramammary treatment is thought to be sufficient to eliminate *S. agalactiae*, but, as demonstrated in a study by Biggs (1996), some herds need a course of treatment. Biggs (1996) used a single dose of an intramammary preparation containing 1g of penicillin G and 0.5g of dihydrostreptomycin (Streptopen MC, Schering-Plough Animal Health, Uxbridge) to successfully eliminate *S. agalactiae* infection from treated herds. Purchased animals should ideally be treated prior to entry into a herd which has been 'blitzed' so as to prevent re-introduction of infection into the herd, unless the herd from which the animals were purchased has a low BMSCC and/or is known to be free of *S. agalactiae* infection (Biggs, 1996).

The economic value of treating late lactation cows is questionable, as it will be unlikely that the yield will improve by a sufficient amount to cover the cost of treatment (Yamagata and others, 1987). However, failure to treat these cows could leave a reservoir of infection in the herd (Blowey and Edmondson, 1995).

Following whole herd or partial herd 'blitz therapy', the milk will need to be withheld for the statutory period, which varies depending on the drug used. During this time the farmer will not receive income from milk. It should be remembered that the milk withdrawal period is yield dependent, therefore in some smaller, low-yielding herds, the threshold used for calculating the length of milk withdrawal may be exceeded (Blowey and Edmondson, 1995). Arrangements for testing the milk for antibiotic residues prior to collection should be recommended (Blowey and Edmondson, 1995).

'Blitz therapy' can fail due to the introduction of new cows into the herd (Roberts, 1969), careless milking hygiene allowing residual infection to be transferred from cow to cow, failing to use DCT on all dry cows and, if a partial 'blitz' regime is being used, failing to identify all infected cows, (Biggs, 1996). Persistence of infection could be due to ineffective treatment of existing cases; whereas new intramammary infections could arise due to poor control or hygiene measures (Blowey and Edmondson, 1995). Good milking routine is essential for the success of 'blitz therapy', as it has been reported that infection levels can return to those present prior to treatment within one or two years if a poor milking routine persists (Biggs, 1996). Hygiene is also important in preventing other pathogens taking the place of *S. agalactiae* following 'blitz therapy' (Roberts and others, 1963). Isolation of *S. agalactiae*, following total 'blitz therapy', indicates inadequate teat disinfection and/or DCT, or the introduction of an infected animal (Bramley, 1992).

The major advantage of 'blitz therapy' is the rapid reduction in the prevalence of infection and SCC compared to the enforcement of a control programme of teat dipping and DCT (Erskine and Eberhart, 1990).

1.9.3. Eradication

Elimination of infection from cows can be achieved by (1) the defence mechanisms of the udder eliminating the pathogen i.e. spontaneous cure, or (2) by treatment during lactation, or (3) by the treatment during the dry period, or (4) by culling infected animals (Bramley and Dodd, 1984). The diligent application of proven control measures reduces the incidence of mastitis due to *Staphylococcus spp.* and *Streptococcus spp.* However, it is possible to eradicate *S. agalactiae* from herds and three options are available. (1) total herd 'blitz therapy', (2) partial herd 'blitz therapy', and (3) improved dairy management (Biggs 1996). Blitz therapy has already been discussed (Chapter 1.9.2.). Biggs (1996) considers *S. agalactiae* too contagious to control by improved management alone. However, Bramley and Dodd (1984) believe it would be uneconomical to strive for eradication of the common major pathogens, even *Streptococcus agalactiae,* due to the cost of laboratory services and because there are now effective control measures which will reduce the level of infection to very low levels. Eradication of one infectious agent is useless if it is followed by a rise in the rates of others. Therefore before carrying out 'blitz' treatment, it is recommended to adopt a scrupulous milking routine and maintain adequate hygiene standards (Roberts and others, 1963). If hygiene is not maintained at an adequate level there is a risk that *S. agalactiae* will be re-introduced into the herd, and as the herd will be completely naive following a 'blitz therapy', the organism would spread rapidly between cows.

Unlike most infections with *Streptococcus* spp., *S. aureus* infections are very difficult to eradicate, which is due to the fact that *Staphylococcus spp.* invades tissue and intramammary infusions may be unable to gain access to the organism (Ziv, 1980). Therefore eradication is often not attempted but a reduction in *S. aureus* infections may follow enforcement of the 'Five Point Plan' and culling of infected cows, where possible.

1.10. Impact

Despite rigorous implementation of the 'Five Point Plan' giving good control of subclinical mastitis by post-milking teat disinfection and DCT, the prevalence of clinical mastitis may remain unaltered (Eberhart and Buckalew, 1977). However, a change in the organisms responsible for the clinical cases may be seen (Erskine and Eberhart, 1988). Environmental pathogens are becoming more important as major causes of bovine mastitis, as the incidence of contagious pathogens reduces (Booth, 1993). Shukken and others (1989) have demonstrated in low BMSCC herds, that teat disinfection after milking increases the percentage of uninfected quarters and increases the risk of clinical mastitis. This may be because teat dipping and DCT are least effective against environmental pathogens (Natzke, 1981; Oliver and Mitchell, 1984) or because the presence of minor pathogens tends to prevent the multiplication of major pathogens (Brooks and Barnum, 1984; Shukken and others, 1989). Reports by Dodd (1983) and by Oliver and Mitchell (1984) have also shown that the prevalence of environmental mastitis has increased, especially in low SCC herds. These authors found that environmental pathogens made up more than eight to nine times the percentage of isolates in *S. agalactiae* negative herds as compared to *S. agalactiae* positive herds. As it is difficult to interpret herd survey data on coliform infections, the prevalence of these is probably underestimated. Miltenberg and others (1996) reported that, in Southern Netherlands, a high prevalence of clinical mastitis is a problem in herds with low BMSCC. These increasing cases of severe mastitis tend to be associated with *E. coli* infections (Wilesmith and others, 1986). This alteration in the relative importance of the organisms associated with clinical mastitis may be due to management changes and adoption of mastitis control measures as *E. coli* and *Streptococcus uberis* infections are more prevalent than 15 years ago (Wilesmith and others, 1986). Therefore, the statement that 85-95% of bovine mastitis cases are caused by *S. aureus* and *S. agalactiae,* should be qualified by adding that this is likely to be the situation in herds which do not carry out post-milking teat dipping or DCT (Eberhart and Buckalew, 1977; Oliver and Mitchell, 1984).

1.11. Prevention

1.11.1. Genetics

There has been an increasing interest in breeding cattle for reduced susceptibility to mastitis (Shook, 1993). A survey by Hibbitt and others (1980) suggested the existence of a relationship between genetics and the prevalence of mastitis. Hamori (1980) demonstrated that cows selected for high dairy performance, had enormous bulky udders and were more liable to incur injuries, and therefore mastitis was more likely. Shook (1993) reported a positive relationship between milk yield and mastitis, suggesting that the genes for increased milk yield also tend to increase the susceptibility to mastitis. Another predisposing factor to the development of mastitis was found to be the conformation of the teat and teat orifice and broad patent teat canals with inadequate sphincter muscles were seen to facilitate ascending invasion of infectious agents (Hamori, 1980). However, the factors allowing teat canal penetration may also be associated with low milk yield and slow milking (Bramley and Dodd, 1984). Shook (1993) recommended the use of SCC as an indicator trait for genetic improvement for mastitis resistance. However, genetic improvement for SCC will not replace the requirement for good herd management to reduce mastitis (Hansen, 1993).

1.11.2. Vaccination

Vaccines against *S. aureus* mastitis are available in the USA. However, these vaccines do not prevent all infections, and if infected cattle remain in the herd, they will act as a reservoir of infection and segregation of infected cows would be necessary (DeGraves and Fetrow, 1993). The bacterin appears to reduce the frequency and severity of clinical episodes of *Staphylococcus aureus* infections, but is unlikely to have any great impact on the incidence or prevalence of infections (Tyler and others, 1993). Given the efficacy of inexpensive antibiotics, and the ease of eradication of *S. agalactiae,* a bacterin for *S. agalactiae* would need to be highly efficacious before its use would be recommended (DeGraves and Fetrow, 1993). It is, therefore, unlikely that commercial vaccines against the main contagious pathogens will become available in the UK in the near future.

1.12. Approach to the investigation of a problem farm

Herd mastitis investigations can be divided into three major stages (David, 1993):

1. Assessment of herd data,
2. The farm visit and,
3. Plan of action, formed after evaluation of herd data and the on-farm situation.

Mastitis is noted as being a problem by the farmer when there is an increase in the incidence of clinical mastitis, or an increase in BMSCC, or an increase in bulk tank TBC or bactoscan, or any combination of these factors (Bushnell, 1980; David, 1993). In addition to these factors, mastitis cases which are slow to clear, repeated periodic bouts of clinical cases, and cases recurring soon after treatment may also encourage the farmer to seek veterinary advice (Watson, 1998). The examination of herd records may indicate whether environmental or contagious pathogens are likely to be the problem (Blowey, 1984; David, 1993). The clinical records are also the most accurate measure of the incidence of mastitis in a herd (David and Jackson, 1984). Repeated clinical incidents in the same cows are suggestive of a chronic *S. aureus* problem, whereas a situation where there are large numbers of cows with new infections suggests environmental pathogen involvement (David, 1993). The time and duration of infection in relation to the stage of lactation are also useful in determining the nature of the infection (Blowey, 1984; David, 1993). Environmental infections tend to be more common in freshly calved cows, whereas contagious pathogens tend to cause mastitis at any stage of lactation (David, 1993). Bacteriological examination of milk samples is one of the most important components of a mastitis investigation (David, 1993), and monthly culture of bulk milk samples is a practical and economical procedure to monitor mastitis at the herd level (McDonald, 1989; Biggs, 1996). Bulk milk culture is also useful as a post-treatment check (Biggs, 1996). The identification of the causal pathogen is fundamental in an investigation of a mastitis problem as it allows areas requiring investigation to be identified, i.e. the environment or the milking plant, and consequently which control measures should be focused on (David and Jackson,

1984). Trends in BMSCC (Jackson, 1980), ICSCC and TBC provide useful means to follow the pattern of infection.

A farm visit to assess the milking machine design and function, and the milking routine, as well as the environment and housing of the cows is important to discover any factors predisposing to mastitis (Bushnell, 1980; Kirk, 1990; David, 1993). The general management of the herd, including stocking density, feeding and grouping, is also important (David, 1993).

After having evaluated the herd records and visited the farm, a decision on how to proceed may be made. Somatic cell counting and bacteriological examination at the cow or quarter level may be necessary. The CMT is a useful technique to make the bacteriological sampling more cost-effective (Bushnell, 1980). The steps taken to combat the problem will differ depending on the type of organism involved. If an environmental pathogen is involved, control measures may include: cleaning out and re-bedding the housing area and improving the drainage and ventilation (David, 1993). Drug treatment may be the intervention of choice for *S. agalactiae*, whereas treatment, segregation or culling may be advised for *S. aureus* (David, 1993).

1.13. Summary

Mastitis remains the most prevalent disease of adult dairy cattle. Subclinical mastitis has increased in significance following the introduction of the EC Health and Hygiene Directive 92/46 in 1994. As milk companies can dictate the standards for milk quality prior to agreeing to purchase milk from the producer, as well as imposing penalties for inferior quality milk, the control of subclinical mastitis is an economic concern for the milk producer. Diagnosis of infectious agents relies upon evaluation of farm practices relating to milking and housing, and bacteriological examination of milk samples to identify the causal organisms. *Streptococcus agalactiae* and *S. aureus* are still the most common isolates in dairy herds with poor hygiene practices. However, environmental pathogens are increasing in significance. The main approach to mastitis control and prevention remains a reliance on good

milking practice and adherence to the 'Five Point Plan', as was the case 30 years ago. There has been increasing interest in vaccinating against mastitis pathogens and in improving genetics so as to increase the resistance of cows to mastitis.

Chapter 2

Investigation of a high somatic cell count dairy herd

2.1. Introduction

High BMSCCs are often associated with subclinical mastitis infections in the udder (Dohoo and Meek, 1982). Contagious mastitis pathogens are more likely to produce subclinical infections, and therefore a high BMSCC, than environmental pathogens (Blowey and Edmondson, 1995). Herd records, especially those recording clinical cases of mastitis, are useful in determining the likely cause of a mastitis problem. The clinical records provide the most accurate measure of the herd incidence of mastitis (David and Jackson, 1984). *Streptococcus agalactiae* and *S. aureus* are the major pathogens associated with subclinical infections in Scotland (Logue and others, 1994; Logue, 1997) and in Massachusetts (Oliver and Mitchell, 1984). Infected cows will have a decreased milk yield, as much as 10%, and there will also be additional treatment and labour costs, should the infection become clinical (Hale, 1956; Winkler, 1982).

When investigating a mastitis problem, after examining herd records, it is important to observe the milking routine used on the farm and check the milking machine (David, 1993). In the diagnostic work-up, somatic cell counting will be useful at the bulk tank, individual cow and/or individual quarter level (Jackson, 1980; Sears, 1993; Biggs, 1996). The CMT crudely identifies high SCC cows and can be used to improve the efficiency of milk sample collection for bacteriological examination, i.e. only quarters giving a positive result on CMT may be sampled for bacteriological examination (Jaartsveld, 1975).

The management of contagious mastitis problems depends on the bacteria involved. For *S. aureus* infections, treatment and eradication are very difficult, and the

approach to a herd infected with *S. aureus* may include segregation and/or culling of infected cows (Wilson, 1980). Intermittent shedding of *S. aureus* complicates the diagnosis of mastitis problems caused by this pathogen (Sears and others, 1993). In contrast, *S. agalactiae* can often be eliminated from herds (Philpot, 1975; McDonald, 1977) as it is sensitive to most antibiotics and can only survive in the environment for a limited period (Plommet and LeLouedee, 1975). Control measures for the eradication of *S. agalactiae*, therefore, usually involve strict adherence to the 'Five Point Plan' (Chapter 1.9.1.), and treatment of infected cows or of the whole herd (Biggs, 1996).

2.2. Farm background

2.2.1. Livestock

This project was based on the investigation of a high SCC dairy herd consisting of sixty cows, mainly Holstein/Friesian and some Ayrshires. The cows were kept on a family-run farm of approximately 114 hectares. In addition to the dairy cows, there were 200 Scottish Blackface ewes, six beef suckler cows and one Blonde D'Aquitaine stock bull kept on the farm. In general, the dairy cows were small in body size and had a long hair coat especially over the udder.

2.2.2. Breeding policy

Artificial insemination (A.I.) was routinely used for all dairy cows, with natural service using the stock bull, being reserved for repeat breeders. Nine or ten heifers were introduced into the herd every year. These dairy replacements were usually home-bred, but, occasionally, calved heifers were purchased to maintain the herd size.

2.2.3. Housing and feeding management

Over the winter months, November to April, the cows were accommodated in cubicles. Dry cows, milking cows and in-calf heifers were housed together in the same cubicle house. The cubicles were in single rows and were not bedded with the cows lying on the concrete bases. The passageway in the cubicle house was scraped

once daily using a tractor and yard scraper. A feed barrier allowed ad-lib access to silage during the housing period. During the housing period, the milking herd received a standard daily ration of 2 kg/cow of BOCM Pauls dairy concentrate (18% crude protein). Recently calved cows received an additional daily concentrate ration at a rate of approximately 2 kg/cow, which was divided and fed in the byre during each milking for at least one month after calving. No additional feed allowances were made for higher milk yields.

2.2.4. The milking machine and the milking routine

The cows were milked in groups of 10 in a byre using a pipeline system. Ten cows were tied by the neck down either side of the byre for the duration of the milking procedure. There was no collecting or holding yard and cows were returned to the cubicle house or the field immediately following milking.

There were no milk jars so the milk was carried directly from the cows into the bulk tank meaning that it was not possible to assess individual cow milk yield. This milking system had been in place for approximately 40 years. Individual components had been replaced over time and the milking machine had undergone annual inspection by technicians from the milk purchasing company. It was standard practice to replace the liners for the clusters every six months.

2.2.5. Herd records and culling policy

Herd records were very poor: important events such as calving dates, cases of mastitis and treatments administered were not recorded. The only form of records kept were the A.I. sheets giving the service dates. Cows were culled based on age, poor reproductive performance and recurrent cases of clinical mastitis.

2.2.6. Mastitis treatment and dry cow therapy

On detection of clinical mastitis the farm policy was to infuse novobiocin, neomycin, procaine penicillin G, dihydrostreptomycin and prednisolone (Tetra Delta, Pharmacia and Upjohn Limited Limited, Corby) intramammary tubes, if the cow failed to respond or was systemically ill then the local veterinarian was called. The DCT used

on the farm was framycetin, penethamate hydriodide, procaine penicillin (Leo Red Dry Cow, Leo Laboratories Limited, Princes Risborough).

2.3. Reason for investigation

Veterinary investigations were initiated on this farm following notification to the farmer by the milk company threatening the possible termination of the milk contract due to persistently high BMSCC. The requirements set by the milk company, during the study period are summarised in Tables 1 and 2.

Somatic cell count	
< 250,000/ml	+ 0.1 ppl
250,000-400,000/ml	NIL
401,000-500,000/ml	- 1.0 ppl
> 500,000/ml	- 3.0 ppl

Table 1: Somatic cell count (SCC) payment bands, based on three month geometric mean.

Hygienic milk quality (TBC)	
< 15,000/ml	+ 0.2 ppl
> 50,000/ml	- 2.0 ppl
> 100,000/ml (first occasion)	- 5.0 ppl
> 100,000/ml (twice or more within 6 months)	- 10.0 ppl

Table 2: Hygienic milk quality bands, based on two month arithmetic mean.

2.4. Materials and methods

2.4.1. Historical data

Geometric monthly BMSCC for two years prior to the start of the investigation, in addition to ICSCC which were recorded for all lactating cows on one occasion by the local veterinary practitioner, were available. These data were consulted and used for planning the approach to the farm investigation.

2.4.2. Initial farm investigations

A farm visit was arranged in December 1996 to observe the milking routine. The ICSCC results from November 1996 were consulted, and cows with ICSCC > 400,000 cells/ml were selected for further investigation during the farm visit in December 1996. Twenty three cows were identified for further investigation on this basis. The CMT was used to identify cows with a high SCC on the day of sampling. The CMT was performed on all four quarters of the selected cows. Quarters which were positive on CMT were sampled for bacteriological examination.

2.4.2.1. General observations

While on the farm the milking routine and general management of the dairy were assessed. This was important to establish a basis for how best to proceed with the investigation. The cows were milked twice daily in a byre using a pipeline system. The byre held 10 cows on each side and five different clusters were used.

2.4.2.2. California Mastitis Test

The CMT relies upon a chemical reaction between the CMT reagent, an anionic surface active agent or detergent and the nucleic acid of somatic cells to produce a gel with bromocresol-violet acting as an indicator (Schalm and Noorlander, 1957; Philpot, 1984; Bramley, 1992; Muldoon, 1995; Radostits and others, 1995a). A gel is formed when large numbers of somatic cells are mixed with the CMT reagent (Schalm and Noorlander, 1957; Philpot, 1984; Bramley, 1992; Quinn and others, 1994; Radostits and others, 1994b; Blowey and Edmondson, 1995; Muldoon, 1995).

The cows to be sampled were prepared as for milking, i.e. dirty udders were washed with water and dried using paper towels. The technique for CMT as described by Schalm and Noorlander (1957), Philpot (1984), Quinn and others (1994), Radostits and others (1994b), Blowey and Edmondson (1995) and Muldoon (1995) was followed. The first five to ten ml of milk from each quarter were discarded. An equal volume of quarter milk and the CMT reagent were placed in each of the four wells of the CMT paddles. The paddles were rocked gently for a few seconds and

observed for any gel formation. Gel formation was considered to be a positive result for high somatic cell content in the milk, and those cows were identified for further examination.

2.4.2.3. Milk samples

Milk samples were collected from quarters which gave a positive CMT result. Sterile glass or disposable plastic containers are considered suitable for sample collection (Higgs and Bramley, 1980; Shotts and Leard, 1984; Bramley, 1992; Blowey and Edmondson, 1995; Cullor and Tyler, 1996). In order to minimise the risk of contamination of milk samples fastidious attention was paid to teat preparation, as described by Higgs and Bramley (1980), Shotts and Leard (1984), Watts (1990), Bramley (1992), Blowey and Edmondson (1995), Buswell (1995), Muldoon (1995) and Cullor and Tyler (1996). The udders and teats were washed and dried using individual paper towels and the teat ends were disinfected with 70% alcohol swabs, prior to collection of milk samples. The alcohol was allowed to evaporate prior to milk collection. The furthest away teats were cleaned first and the nearest teats were cleaned last with the milk samples being collected in the opposite order, to reduce the chance of contamination by the milk collector. The first four to six squirts of milk (approximately 10 ml) were discarded to avoid contamination of the milk sample by bacteria from the teat canal. When collecting the milk, the container was held as close to horizontal as possible and the lid of the container was held face down in the same hand in order to minimise the entry of any contaminants and the container was only filled to two thirds of its capacity. Approximately 10-15 ml is considered to be an adequate volume from each positive quarter for bacteriological culture and sensitivity. In between sampling cows, the sampler washed his/her hands with a germicidal solution and, used a new pair of clean disposable gloves for each cow.

2.4.2.3.1. Bacteriological examination

The milk samples were transported directly to the laboratory as recommended by Bramley (1992). It is recognised that most bacterial pathogens causing mastitis grow

on sheep blood agar (Shotts and Leard, 1984; Bramley, 1992; Quinn and others, 1994). Other media commonly used included MacConkey, horse blood and chocolate agar medium. The MacConkey agar is used to allow *Enterococccus faecalis* and some gram-negative bacteria to grow (Watts, 1990; Quinn and others, 1994). Horse blood agar is used to culture anaerobic bacteria. The chocolate agar is used to aid differentiation of bacteria by means of haemolysins.

The chocolate agar plates were incubated in 50% carbon dioxide and the horse blood agar plates were incubated under anaerobic conditions using nitrogen and hydrogen gases.

A standard sterile 7mm bacteriological wire loop was used to streak the agar plates, as described by Higgs and Bramley (1980), Shotts and Leard (1984), Quinn and others (1994), Buswell (1995) and Radostits and others (1995b). The plates were incubated at 37°C and examined at 24 and 48 hours after inoculation, as recommended by Bushnell (1980), Higgs and Bramley (1980), Shotts and Leard (1984), Bramley (1992), Quinn and others (1994), Blowey and Edmondson (1995), Buswell (1995) and Radostits and others (1995b). The presence of colonies was noted and the colonies were then examined. To identify *Streptococcus spp.* the commercial latex agglutination kit, the API (Analytab Products or bioMerieux) system (Quinn and others, 1994) or the CAMP test were used (Bushnell, 1980; Shotts and Leard, 1984; Watts, 1990; Cullor and Tyler, 1996). The CAMP test is where a *S. aureus* capable of producing a large zone of partial haemolysis is streaked across the centre of blood agar and a colony of *Steptococcus spp.* is streaked perpendicular to the line of staphylococci. The plate is then incubated and examined after approximately 24 hours for evidence of a semi-circular or arrowhead shaped zone of complete haemolysis in the zone of partial haemolysis, which would be a positive result of the CAMP test indicating that the *Streptococcus spp.* was *S. agalactiae* (Higgs and Bramley, 1980, Quinn and others, 1994). Gram-stained smears of any colonies were examined microscopically, as recommended by Watts (1990) and Buswell (1995), to detect whether the organism was gram-positive or gram-negative.

The procedure for testing antibiotic sensitivity has been described by Blowey and Edmondson (1995) and their methods were followed in this study. In order to test for antibiotic sensitivity, single colonies were taken from the original agar plate and added to liquid media, incubated for six to eight hours, and then poured over the surface of a second agar plate before small paper discs, impregnated with different antibiotics, were placed on the surface of the plate. This plate was then incubated for a further 24 hours. The zone of inhibition around the antibiotic discs were measured and compared to a standard as described by Prescott and Baggot (1993). Under crude interpretation this means that if bacterial growth was inhibited around a disc, these bacteria were considered to be sensitive to that antibiotic *in vitro*. However, if the bacterial growth reached the edges of an antibiotic disc, the bacteria were considered to be resistant to that antibiotic. The antibiotics tested included ampicillin, enrofloxacin, chloramphenicol, lincomycin, oxytetracycline, penicillin, streptomycin, trimethoprim/sulphonamide, amoxycillin and clavulanic acid, neomycin and cephalexin. The antibiotics for sensitivity were the laboratories standard test set.

2.4.2.3.2. Bacterial identification

Staphylococcus aureus colonies tended to be round, white-yellow in colour and surrounded by a double zone of haemolysis on sheep blood agar (Higgs and Bramley, 1980; Shotts and Leard, 1984; Quinn and others, 1994; Blowey and Edmondson, 1995; Buswell, 1995). This bacteria did not grow on MacConkey agar. Microscopic examination of a gram-stained smear revealed gram-positive cocci (Higgs and Bramley, 1980; Quinn and others, 1994; Blowey and Edmondson, 1995). A coagulase test was used to confirm that the isolate was coagulase positive and therefore *S. aureus* (Higgs and Bramley, 1980; Shotts and Leard, 1984; Quinn and others, 1994; Buswell, 1995) and a catalase test was used to differentiate staphylococci from streptococci and enterococci (Watts, 1990; Quinn and others, 1994).

Streptococci spp. colonies were small, translucent with α, β or γ haemolysis on sheep blood agar (Higgs and Bramley, 1980; Shotts and Leard, 1984; Quinn and others, 1994; Blowey and Edmondson, 1995; Buswell, 1995). α haemolysis: a zone of greening or of partial haemolysis. β haemolysis: a clear zone of haemolysis. γ haemolysis: no haemolysis (Higgs and Bramley, 1980; Shotts and Leard, 1984). Most *Streptococci spp.* do not grow on MacConkey agar, except some Lancefield group D streptococci and *E. faecalis* (Quinn and others, 1994). The CAMP test was used to identify *S. agalactiae*, which enhanced the partial haemolysis of *S. aureus* on sheep blood agar (Higgs and Bramley, 1980; Shotts and Leard, 1984; Quinn and others, 1994). Streptococci were divided into different groups according to C-substance which is a group specific cell wall polysaccharide. Grouping was carried out using the API system (Quinn and others, 1994). Microscopic examination of a gram stained smear revealed gram-positive cocci (Blowey and Edmondson, 1995).

2.4.3. Subsequent farm investigation

A second farm visit was arranged in January 1997 in order to collect samples for bacteriological examination from cows which had previously given a negative result on CMT, and from cows which were identified to have low ICSCC in November 1996. These cows were selected in order to determine how widespread the mastitis infections were even in cows with low ICSCC, and to aid in decision making on how best to manage the problem on this farm. Milk samples were collected from all four quarters of the selected cows for bacteriological examination, as described in Chapter 2.4.2.3.

2.4.3.1. Bacteriological swabbing

Swabs for bacteriological examination were taken from the cubicles, water troughs, surfaces in the byre, udder washing bucket, cluster liners and cow's udders in order to identify possible environmental sources of udder infection. The swabs were used to streak the agar plates and these were incubated and examined as described for the milk samples (Chapter 2.4.2.3.1.).

2.4.4. Data storage and analysis

Data from all phases of this study were stored in a Microsoft Access (Microsoft Corporation) version 2.0 database running on a Viglen Genie PCI Professional Microcomputer (486, 266 MHz microcomputer,1Gb hard disk).

2.4.4.1. Database design

Microsoft Access (Microsoft Corporation) is a relational database management system that allows the storage of data in related tables. A **table** is designed by naming and specifying the properties of *fields* (columns) which usually relate to one piece of information for each cow, such as calving date. Field properties relate to the format of data held in the field such as number, text or date. Properties can be specified that limit the permissible entries in a field, which can be a useful way of reducing errors in data entry. Each cow occupies a single row of the table with an entry in all appropriate columns. A *key field* (or fields) may be defined to identify uniquely each record for the purposes of indexing: in this study the key field was defined as the cow identification number. Fields common to two tables may be used to *relate* these tables for the purposes of **querying** across multiple tables. Single or multiple tables can be queried by specifying *criteria* and writing *functions* to limit and aggregate the data in any desired way. A **form** shows one complete record on screen at a time. As they can be made to follow a logical pattern, forms are a useful medium for data entry. Wherever possible, responses were coded to avoid time consuming typing and reduce data entry errors. Simple descriptive data analyses were carried out by means of queries. A sample form is presented in Appendix 1.

2.4.4.1.1. Data analysis

Where appropriate, analysis of variance (ANOVA) and multiple range tests (Newman-Keuls) were used to assess the statistical significance of changes in ICSCC following implementation of control measures (Pagano and Gauvreau, 1993).

2.4.5. Costings

This study was performed on a commercial farm and it was hoped that the method used would be a useful and practical option for veterinary surgeons in general

practice. The cost of diagnostic testing and materials used were estimated and considered for every part of this project. Time and labour were provided free of charge by veterinary researchers and students.

The standard charge for bacteriological examination of a biological sample at the University of Glasgow, Department of Veterinary Bacteriology was £10, irrespective of whether it was a milk sample or a bacteriological swab.

The retail value of a one litre container of CMT reagent was £3.70 and assuming 5 ml of the reagent was mixed with the same volume of milk this volume would be sufficient for 50 cows, it would cost approximately 8p per cow to test all four quarters with CMT reagent.

The Scottish Agricultural College in Auchincruive charge 50p per sample for somatic cell counting.

2.5. Results

2.5.1. Historical data

The geometric monthly BMSCC for the two years prior to the start of the investigation are summarised in Figure 1. The figure is set out in accordance with quota years which ran from the beginning of April to the end of March. These monthly BMSCC data are presented in full in Appendix 2.

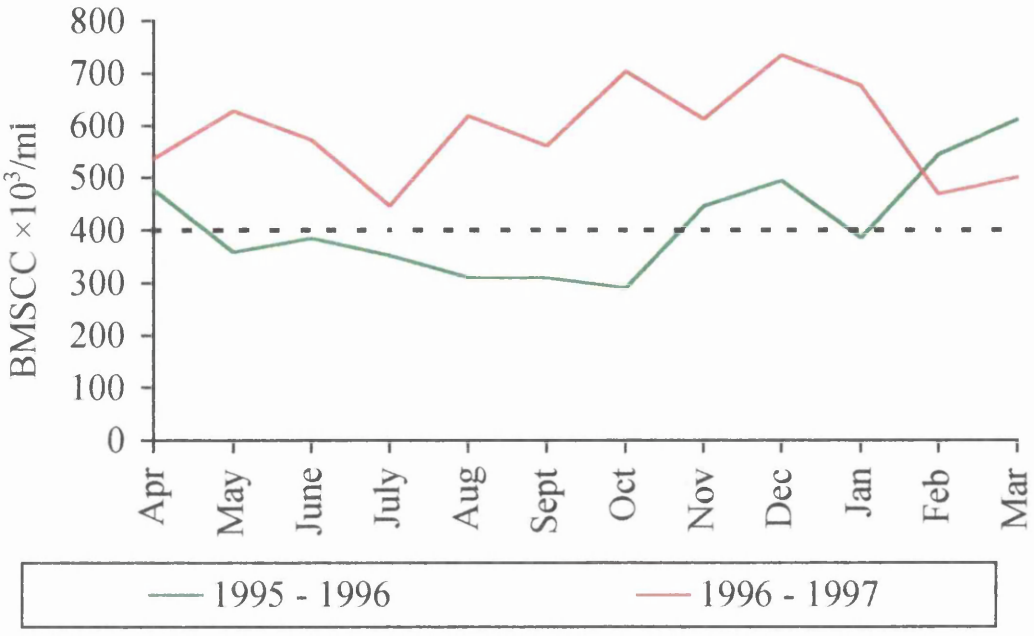


Figure 1: Bulk milk somatic cell counts (BMSCC) for two quota years (1995-1996 and 1996-1997) prior to investigation of the mastitis problem.
----- denotes the maximum BMSCC acceptable under the EC Health and Hygiene directive 92/46.

It can be seen that the BMSCC was increased above the standard three month geometric mean given by EC directive 92/46 of 400,000 cells/ml from November 1995 through 1996 to the start of the study in November 1996. Throughout 1996 the BMSCC fluctuated, but was consistently above the maximum acceptable level. In December 1996 the BMSCC reached a peak of 735,000 cells/ml.

Individual cow somatic cell counts recorded in November 1996 are summarised in Figure 2.

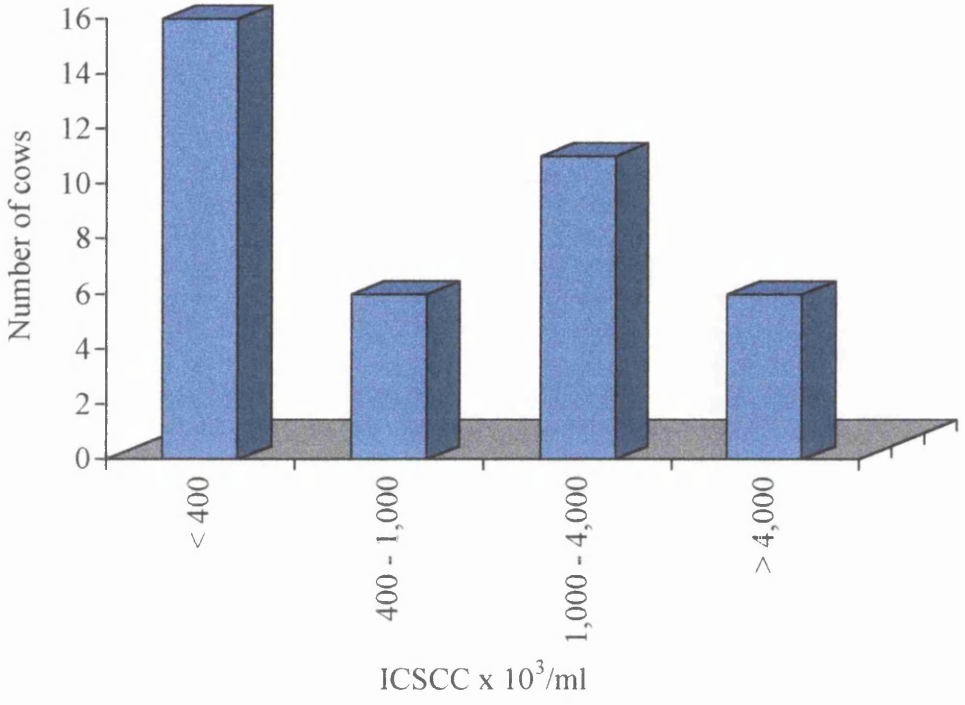


Figure 2: Individual cow somatic cell counts (ICSCC) in November 1996.

Thirty nine cows were in milk at the time of sampling. Twenty three were found to have an ICSCC greater than 400,000 cells/ml, 17 had an ICSCC greater than one million cells/ml, one cow had an ICSCC greater than 18 million cells/ml and only 16 cows had an ICSCC less than 400,000 cells/ml. These ICSCC data are presented in full in Appendix 3.

2.5.2. Initial farm investigation

2.5.2.1. General observations

In general the buildings were in a poor state of repair. The cubicle house walls were made of corrugated iron sheets and several of these were loose and the lying areas were covered in faeces. The individual cubicles were approximately 1.8 metres long by 1.2 metres wide and the step was very variable but was approximately 20 cm high. However, the byre was maintained in good condition and appeared to be cleaned regularly.

Two people were involved in the milking process. Udders which were considered to be dirty by the staff were washed using bare hands with warm water from a bucket; often the same water was used to wash ten cows' udders. The water was only changed after washing cows on one side of the byre. The milkers did not clean their hands during the milking process. Udders which were washed were subsequently dried using disposable paper towels, one for each cow. Foremilking was not carried out. Instead udder palpation during the washing procedure was relied upon to detect cases of clinical mastitis. Post-milking teat dipping was not practised. There was no holding or collecting yard for the cows to gather and the cows were returned to the cubicles or field immediately after milking. Routine DCT was not practised, largely due to the fact that calving dates were unknown. The length of the dry period was very variable and occasional antibiotic failures at bulk milk sampling had been a problem in the past.

The cleaning of the milk line was also unsatisfactory. The cleaning routine after milkings differed in the afternoons compared to the mornings. In the mornings, the pipeline was cleaned using a cold water rinse, followed by circulation of hot water plus detergent followed by a final cold water rinse. In contrast, in the afternoons only a cold water rinse was used.

It was advised that hot water should be used to clean the milk line in the afternoon as well as in the morning. Ideally the cleaning procedure should consist of a three phase process; hot rinse, wash and sanitise. However, to encourage farmer compliance and effect an improvement, it was decided to recommend only a hot water wash plus sanitation with sodium hypochlorite.

The liners useage or number of milkings for each liner, in this 60 cow dairy herd which milked twice daily, changed the liners every six months and used five clusters, was 4,320 milkings. Recommendations suggest that liners should be changed after 2,500 milkings or six months, whichever comes first (Blowey and Edmondson, 1995).

2.5.2.2. California Mastitis Test

Of the 23 cows selected on the basis of ICSCC of > 400,000 cells/ml in November 1996, 15 were positive on CMT in one or more quarters. Twenty six quarters from the 15 cows were CMT positive. Four of the cows selected were found to be CMT negative in all four quarters. The remaining four cows which had ICSCC > 400,000 cells/ml in November 1996, were dry at the time of sampling in December 1996 and were, therefore, not available for CMT. The results of the CMT are summarised in Figure 3.

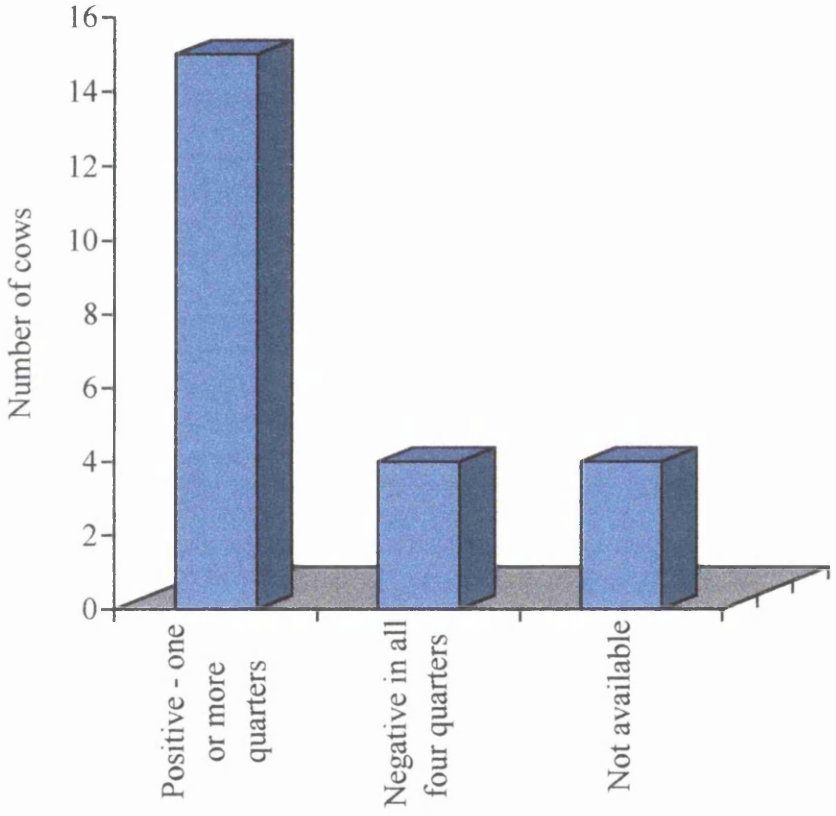


Figure 3: California Mastitis Test (CMT) results from the 23 selected cows.

2.5.2.3. Bacteriological examination

Streptococcus agalactiae was the most common bacterial pathogen isolated from CMT positive quarters, it was isolated from eight cows in total. *Staphylococcus aureus* was isolated from five cows in total. A mixed *S. agalactiae* and *S. aureus* infection was identified in two cows. Other bacteria isolated from these cows, and the other four CMT positive cows included *Staphylococcus epidermidis,*

Corynebacterium spp., *Bacillus* spp. and *A. pyogenes*. Only one CMT positive cow had no bacteria detected in the milk sample taken. Figure 4 shows the number of cows infected with each bacterial isolate.

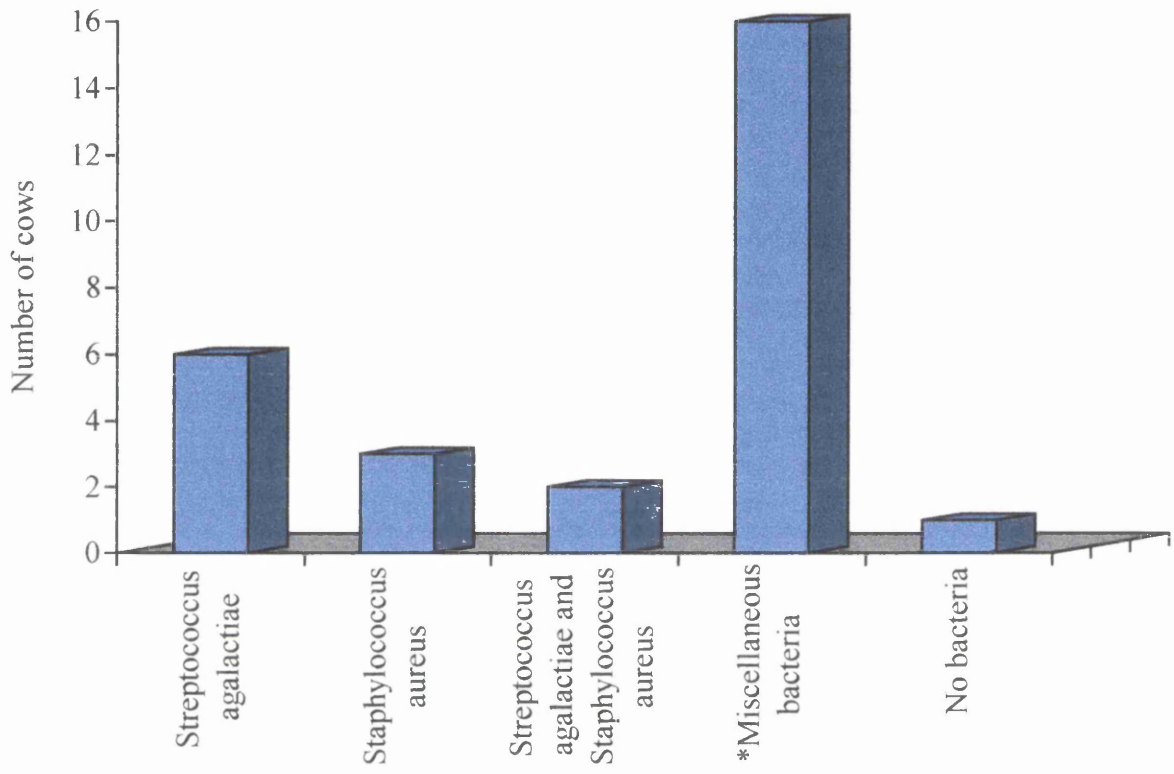


Figure 4: Bacteria isolated from California Mastitis Test (CMT) positive cows. *Miscellaneous bacteria were *S. epidermidis*, *Corynebacterium* spp., *Bacillus* spp. and *A. pyogenes*.

From the 15 CMT positive cows, 26 quarters were CMT positive and were sampled for bacterial culture and sensitivity. *Streptococcus agalactiae* infection was found in 14 quarters, *S.aureus* in six quarters, mixed *S. aureus* and *S. agalactiae* infection in three quarters, and various combinations of the miscellaneous bacteria listed above were isolated from nine quarters. In only one CMT positive quarter were no bacteria detected in the milk sample. Figure 5 shows the number of quarters infected with each bacterial isolate.

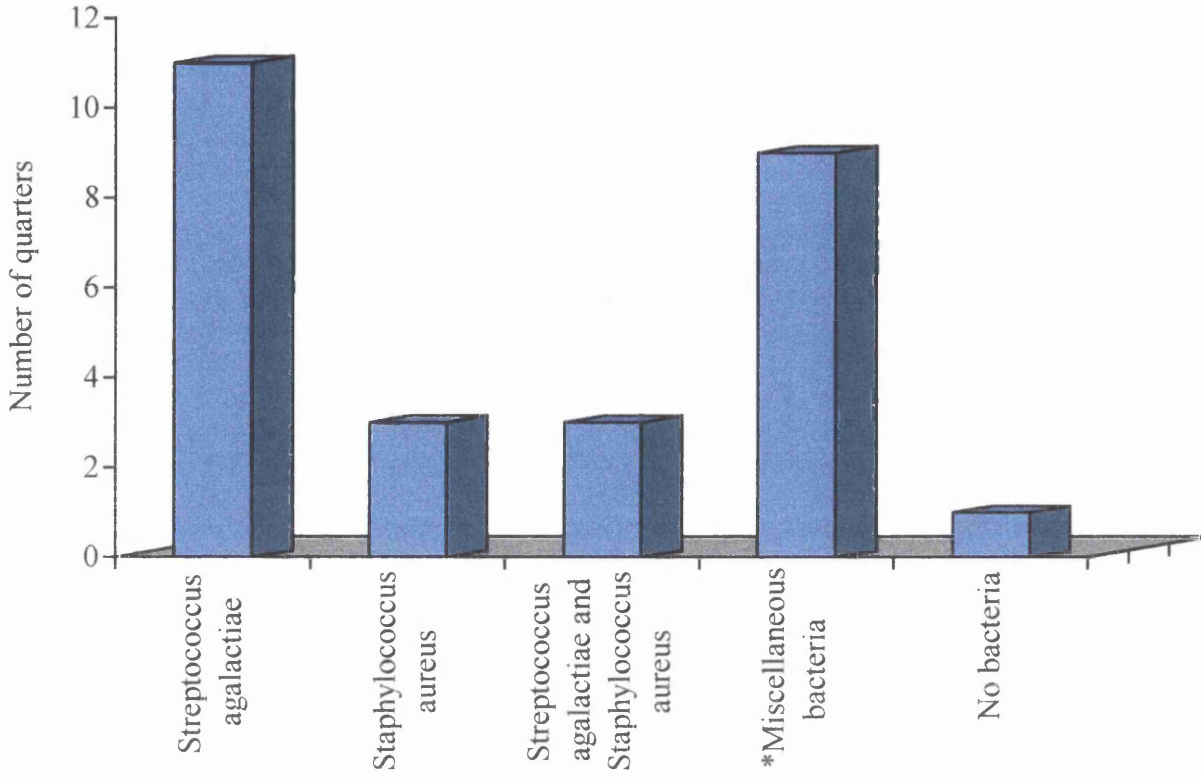


Figure 5: Bacteria isolated from California Mastitis Test (CMT) positive quarters. *Miscellaneous bacteria were *S. epidermidis*, *Corynebacterium* spp., *Bacillus* spp. and *A. pyogenes*.

Antibiotic sensitivities showed that *S. aureus* and *S. agalactiae* isolated from this herd were sensitive to most antibiotics. Table 3 summarises the antibiotic sensitivities of the bacteria isolated.

Antibiotics	*S. aureus*	*S. agalactiae*
ampicillin	some resistance	sensitive
enrofloxacin	sensitive	sensitive
chloramphenicol	sensitive	sensitive
lincomycin	sensitive	sensitive
oxytetracycline	some resistance	sensitive
penicillin	some resistance	sensitive
streptomycin	resistant	resistant
amoxycillin/clavulanic acid	sensitive	sensitive
trimethoprim/sulphonamide	sensitive	some resistance
cephalexin	sensitive	sensitive
neomycin	sensitive	some resistance

Table 3: Antibiotic sensitivities for *Streptococcus agalactiae* and *Staphylococcus aureus* from the study herd.

From these results, *S. aureus* and *S. agalactiae* from this herd were considered to be sensitive to enrofloxacin, chloramphenicol, lincomycin, amoxycillin/clavulanic acid and cephalexin *in vitro*.

2.5.3. Subsequent farm investigation

Two of the cows which were CMT negative at the previous visit had been dried off by January 1997 and so were unavailable for sampling. This left only two CMT negative cows and the four low SCC cows available for sampling. All six cows were found to have *S. agalactiae* infection in one or more quarters, and four of them also had *S. aureus* infection. At the quarter level, six quarters had *S. agalactiae* infection and four quarters had *S. aureus* infection. In these, and other, quarters from the six cows, miscellaneous bacteria were also isolated and included *S. epidermidis, A. bovis* and *Bacillus* spp. No bacteria were isolated from only seven quarters of these six cows.

Bacillus spp. were cultured from swabs taken from the cubicles, water troughs, surfaces in the byre, udder washing bucket and the udder. *Streptococcus agalactiae* was isolated from a swab taken from one of the cluster liners. No other mastitis pathogens were detected from the environmental samples.

2.5.4. Costings

The costs involved in the investigation of the high BMSCC in this herd were the cost of the CMT and the cost of bacteriological examination of milk samples from quarters which were positive on CMT. The cost of the ICSCC, which were carried out by the local veterinary practitioner were included.

Nineteen cows were available for CMT, and 15 were positive, giving a total of 26 quarters which were sampled for bacteriological examination. On the second farm visit, all four quarters of six cows was sampled for bacteriological examination and six swabs were taken from the environment. Therefore, the cost of CMT was £1.52 and the cost of the bacteriological examinations were £560. The ICSCC for 39 cows cost £19.50. Giving a total cost of investigations of £581. Table 4 details the costs of the investigation.

Analysis	Type of sample	No. of samples	Cost of analysis	Calculation	Total
ICSCC	Composite from all lactating cows in November 1996	39	50p per sample	39 × 0.5	£19.50
CMT	All quarters from selected cows	19	8p per cow	19 × 0.08	£1.52
Bacteriology	Quarter samples from CMT positive quarters	26	£10 per sample	10 × 26	£260
Bacteriology	Quarter samples from all cows sampled on second farm visit	24	£10 per sample	10 × 24	£240
Bacteriology	Swabs from environment	6	£10 per sample	10 × 6	£60
TOTAL COST					£581

Table 4: Costs of initial investigation of the herd

2.6. Discussion

Farm production data were limited, meaning that it was difficult to assess the extent of the mastitis problem on this farm. However, historical data for BMSCC were available for two years prior to the investigation of this problem. From these data it could be seen that the BMSCC of this herd had been above the EC standard of 400,000 cells/ml from November 1995, and remained consistently high until the start of this investigation in November 1996. The samples collected in November 1996 showed that 23 of the 39 cows lactating at that time had ICSCC > 400,000 cells/ml. The BMSCC and ICSCC provided sufficient information to identify that this farm had a chronic widespread mastitis problem.

Following the farm visit, an impression of the management and hygiene on the farm was made. The cubicles were too small for the cows and the hygiene was poor due to the lack of bedding and attention to detail. The recommended size of cubicles for modern dairy cows is 2.3-2.4 metres long by 1.2 wide (Blowey and Edmondson, 1995) and the International Dairy Federation (IDF) (Anon, 1987) states that short stalls can increase the incidence of udder disease and that bedding material should be used to provide a clean, dry area for the cows to lie.

It was found that the milking routine and teat preparation were inadequate in relation to the standard recommendations outlined in the 'Five Point Plan' (Philpot, 1984; Bramley, 1992; Biggs, 1996). The washing of the udders from a communal bucket acted as a potential medium for spread of infection between cows. The hands of the staff acted as an additional potential source of contamination (Bushnell, 1984; McDonald, 1984; Fox and Gay, 1993; Radostits and others, 1994a; Rebhun, 1995; Cullor and Tyler, 1996). No foremilking was performed, potentially allowing mastitic milk to enter the bulk tank. Post-milking teat dipping and dry cow therapy were not carried out as a routine, meaning that control and elimination of mastitis pathogens was also likely to be very poor.

The poor cleaning of the milk line may have contributed to the overall problem on this farm. The cleaning routine used was unlikely to be sufficient, as cold water congeals any residual butterfat in the system, thus making it more difficult to remove during the cleaning process (Guterbock and others, 1984). The IDF states that for circulation cleaning, as used on this farm, the water temperature should reach a minimum of 85°C (Anon, 1987). It is known that poor sanitation increases the number of bacteria colonising the pipelines (Guterbock and others, 1984).

The liners were not changed frequently enough. Recommendations suggest that liners should be changed after 2,500 milkings or six months, whichever comes first (Blowey and Edmondson, 1995). Worn liners can cause damage to the teats and can also become more difficult to clean (Blowey and Edmondson, 1995).

The lack of treatment records and the absence of facilities to measure individual cow milk yields meant that it was difficult to assess whether the high BMSCC was due to a small number of cows or whether it was likely to be a herd problem, as a high BMSCC could result from a small number of cows with high ICSCCs (Dohoo and Meek, 1982). Alternatively, the high BMSCC may have been due to a high proportion of cows being in late lactation or having calved recently (Chapter 1.4.2.). The expected calving dates were not known on this farm. For this reason, efficient drying off could not be performed. The dry periods would have been of a variable length and antibiotic residues in milk due to inappropriate DCT would have been likely.

Milk sampling undertaken in December 1996 revealed 14 out of the 21 cows sampled (66.6%) to have *S. agalactiae* infection and nine cows (42.9%) to have *S. aureus* infection. Six cows (28.6%) had both infections. The use of the CMT, along with the ICSCC, was an easy, inexpensive method to determine the best cows to select for further investigations. This method, described by Logue (1997), has been shown to improve the efficiency of bacteriological examination of milk samples. In this study, only one milk sample, from one udder quarter, was found to be sterile on bacterial culture. The isolation of *S. agalactiae* from a cluster liner suggested that this species

of bacteria may persist in the environment for a long enough period to act as a potential source for the spread of infection within herds, in contrast to published results of others (McDonald, 1977; Biggs, 1996; Cullor and Tyler, 1996).

The antibiotic sensitivities showed that the *S. aureus* isolated from this farm were, in general, resistant to the β-lactam penicillins as would be expected from previous reports (Sandholm and others, 1990; McKellar, 1991; Vestweber and Leipold, 1993) and also because the farmer was likely to have poor antibiotic management judging by the geaneral farm management and record keeping. The *S. agalactiae* isolated from this farm were sensitive to most antibiotics with activity against gram-positive organisms, again as would be expected (Prescott and Baggot, 1993).

The cost of investigations, which did not include professional time or farm labour, amounted to £281. Fetrow (1980) reported that farmers are often unaware of the costs of subclinical mastitis and this proved to be true in this farm. The farmer considered the costs of the investigations too high to be cost-effective.

Taking into consideration historical farm data and the results of farm investigations, this farm was found to have a major contagious mastitis problem. At the time of sampling, imminent legislation changes, penalties imposed by the milk purchasers, lost milk yield and poor cow welfare demanded that immediate action was taken to rectify the situation in order to prevent the farm going out of business.

The first step was to implement improvements in the milking routine, with specific mastitis therapy for the herd being considered depending on the uptake of these changes. It was decided that the milking routine should be improved in the first instance in order to assure, as far as possible, the success of any subsequent control measures.

The recommended improvements included:

1. A pipeline for running water should be installed to reach the byre, with a hose attachments fitted, and this is to be used for washing the udders.
2. The washed udders should continue to be dried using individual paper towels for each cow.
3. A strip cup should be used for foremilking to aid in the diagnosis of clinical mastitis.
4. The clusters should be disinfected between milking either side of the byre, when the clusters fall off the cow during the milking process, and especially after milking a mastitic cow.
5. Post-milking teat dipping should be carried out after each milking using an iodine based preparation.
6. The cleaning of the milk line should be improved, such that the routine should consist of washing with hot water and sodium hypochlorite and then a cold rinse for both the morning and evening milkings.
7. Herd records should be improved; service and calving dates, cases of mastitis and treatments administered should be recorded. Charts were given to the farmer to enable the calving dates to be calculated from the service dates and recording sheets were given to aid in the recording of service dates, calving dates and any mastitis cases and the treatments administered. Samples of these forms are presented in Appendices 4 and 5, respectively.

Interpretation of the results suggested that the high BMSCC was largely caused by infection with *S. agalactiae* and *S. aureus*, with other pathogens playing a minor role. Due to the imminent financial problems facing the farmer, a rapid reduction in SCC was thought to be essential. Following discussion with clinicians, bacteriologists, pharmacologists and epidemiologists (Dr. J. Fitzpatrick, Dr. D. Taylor, Professor Q. McKellar, Dr. D. Mellor, Mr T. King and Mr D. Barrett), the decision was made to treat this herd. Consultation of the literature in combination with the knowledge of this expert panel suggested that 'blitz therapy' had been a successful method for eliminating *S. agalactiae* from infected herds (Biggs, 1996; Cullor and Tyler, 1996). With this in mind, and considering the likely relative merits of partial and whole herd

programmes (Chapter 1.9.2.) on this farm, it was decided to opt for a whole herd 'blitz therapy'. The considerations, drug choices, implementation and results of the whole herd 'blitz therapy' are presented in Chapter 3. It was recognised that the presence of *S. aureus* infection would be a complication in the successful reduction of the BMSCC of this herd.

Chapter 3

Whole herd 'blitz therapy'

3.1. Introduction

'Blitz therapy' is the simultaneous treatment of all four quarters of the cows udder with intramammary infection, or all cows in a herd where a high prevalence of intramammary infection has been identified (Roberts and others, 1969; Erskine and Eberhart, 1990; Blowey and Edmondson, 1995; Biggs, 1996) and usually relates to treatment with intramammary preparations (Blowey and Edmondson, 1995). This programmed treatment with antibiotics is aimed specifically at eradicating *S. agalactiae* infection from a herd (Weaver and others, 1986; Erskine and Eberhart, 1990; Blowey and Edmondson, 1995; Biggs, 1996). Whole herd treatment is recommended if the infection has a high prevalence within the herd, if the infection has been present for years as indicated by a prolonged high BMSCC, or if a large proportion of the cows are in milk at the time of instigation of the regime (Biggs, 1996). The whole herd approach is simple to carry out and produces more predictable results than the partial herd approach, although there are greater initial costs involved in treating and discarding milk from all cows (Biggs, 1996). In contrast, the partial herd approach involves more commitment on the farmers' behalf, with repeated herd screening being necessary (Biggs, 1996).

Intramammary administration is considered an adequate route for treatment against *S. agalactiae* infection (Prescott and Baggot, 1993; Blowey and Edmondson, 1995). However, the intracellular nature of *S. aureus* infection means that the intramammary route may not be effective due to poor distribution of the drug into abscesses, or poor uptake into the relevant cellular compartments (Craven and Anderson, 1979; Ziv, 1980; Madgwick and others, 1989; Sandholm and others, 1990; Bramley, 1992;

Blowey and Edmondson, 1995). Parenteral administration with an appropriate antibiotic should achieve better udder distribution and penetration than intramammary administration (Prescott and Baggot, 1993), depending on lipid solubility, degree of ionisation and the extent of protein binding of the drug molecule (Ziv, 1980; Sandholm and others, 1990). Owens and others (1988) reported that intramammary infusion coupled with parenteral treatment was a superior method for treating subclinical mastitis during lactation, compared to using only intramammary treatments.

There are a vast selection of antibiotics licensed for the treatment of mastitis in cattle (Anon, 1998). Both the major bacteria isolated from cows on this farm were gram-positive organisms, therefore, drugs with good efficacy against this type of bacteria were considered for possible use in the treatment of this herd.

The drug of choice for the elimination of *S. agalactiae* infection is penicillin (Bushnell, 1980; Sandholm and others, 1990; McKellar, 1991), which is bactericidal in action. However, penicillin is less effective against *S. aureus* because many strains of this organism produce β-lactamase which renders them resistant to penicillin (Sandholm and others, 1990; Blowey and Edmondson, 1995). Amoxycillin is a broad spectrum penicillin derivative with a better action against *S. aureus* than penicillin (Blowey and Edmondson, 1995). Clavulanic acid is a synthetic drug which brings about the irreversible inhibition of β-lactamase (Sandholm and others, 1990; McKellar, 1991). It is combined in commercial preparations with amoxycillin to produce a drug with a broad spectrum of activity and good action against β-lactamase producing *S. aureus* (Sandholm and others, 1990; McKellar, 1991; Blowey and Edmondson, 1995). Cloxacillin and nafcillin are potentiated penicillins with even better activity than amoxycillin against *S. aureus* and they are also effective against β-lactamase producing strains (McKellar, 1991; Blowey and Edmondson, 1995). However, cloxacillin, nafcillin and amoxycillin are not as efficacious as penicillin against *S. agalactiae* (Sandholm and others, 1990; McKellar, 1991).

Cephalosporins are semi-synthetic, bactericidal antibiotics with good activity against gram-positive bacteria, though less so than penicillins, and all are resistant to β-lactamase (McKellar, 1991; Blowey and Edmondson, 1995). There are no milk withdrawal period for some preparations of cephalosporins, but they are relatively expensive compared to alternative antibiotics.

Macrolides are bacteriostatic antibiotics with good tissue distribution, high intracellular concentrations and activity against gram-positive bacteria and *Mycoplasma* spp.. Erythromycin is a macrolide with good activity against *S. aureus* (McKellar, 1991; Blowey and Edmondson, 1995) but, unfortunately, only intramammary preparations are currently available in the UK for use in cattle (Anon, 1998). Tylosin is a macrolide antibiotic licensed for use in cattle and again is only available as a parenteral preparation (Anon, 1998). Unfortunately, most other macrolides given systemically have long milk withdrawal periods making them unsuitable for use in lactating cows.

The bacteriostatic antibiotics rely on the cow's own defence mechanisms to overcome infection whereas, bactericidal antibiotics kill the bacteria. Therefore, in sick cows bactericidal antibiotics may be preferred. However, the sudden bacterial death may release endotoxins which may make the cow's condition worse (Blowey and Edmondson, 1995).

A sixty cow dairy herd was found to have a high prevalence of *S. agalactiae* and *S. aureus* infections (Chapter 2). *Streptococcus agalactiae* was present in 66.6 % of a pre-selected sample population, and as elimination of this pathogen is possible (McDonald, 1977; Edmondson, 1989; Blowey and Edmonson, 1995; Biggs, 1996), it was decided to start by tackling the *S. agalactiae* infection. Treatment to eradicate *S. agalactiae* infection from the herd was anticipated to bring about a rapid reduction in BMSCC, as shown by Erskine and Eberhart (1990).

3.2. Materials and methods

3.2.1. Therapeutic regimes

Various regimes for the herd in this study were considered and these included:

1. 'Blitz' treatment of all cows against *S. agalactiae.*
2. 'Blitz' treatment of cows known to be infected with *S. agalactiae.*
3. 'Blitz' treatment of all cows against *S. agalactiae* and *S. aureus.*
4. 'Blitz' treatment of cows known to be infected with *S. agalactiae* or *S. aureus.*
5. Any of the regimes 1-4 plus parenteral treatment for *S. aureus.*
6. Any of the regimes 1-4 plus culling of the cows infected with *S. aureus.*

The farm economics and the high percentage of cows infected with *S. aureus* made culling on the basis of *S. aureus* infection impractical. After considering the high prevalence of infection and the chronic nature of the problem within the herd, as well as the ease of management and the more predictable results from whole herd treatment, a whole herd approach was adopted as described by Biggs (1996). The decision to combat the *S. agalactiae* problem using a whole herd 'blitz therapy' with parenteral and intramammary treatment was made after consultation with an expert panel (Chapter 2.10.2). In this herd, the combined use of intramammary and parenteral preparations was chosen to ensure optimal tissue penetration and distribution in order to combat intramammary infection. In this project, although the 'blitz therapy' was aimed primarliy at eradicating *S. agalactiae* from the herd, it was hoped that a reduction in the level of *S. aureus* infection might also be achieved.

3.2.2. Antibiotic options

Antibiotics were considered in relation to their action against both *S. agalactiae* and *S. aureus.* In this study, subclinical mastitis was the major problem and therefore the choice between bactericidal and bacteriostatic antibiotics was not crucial.

After considering the activity, the cost, and the milk withdrawal periods of the drugs, the ideal treatment was considered to be nafcillin or cloxacillin by parenteral administration and penicillin by intramammary infusion. This would have action

against *S. agalactiae* (penicillin) and *S. aureus* (nafcillin or cloxacillin). Unfortunately, there were no preparations available containing cloxacillin or nafcillin for parenteral administration (Anon, 1998), therefore, it was decided to use penicillin by the parenteral route in addition to nafcillin by intramammary infusions. Nafcillin was chosen as the intramammary drug treatment for the lactating cows because of its action against *S. aureus*, and penicillin was chosen as the parenteral treatment for its known efficacy against *S. agalactiae* (Murphy and Stuart, 1954).

The dry cows were also to be treated, as *S. aureus* and *S. agalactiae* are known to persist during the dry period (Dodd and Griffin, 1975; Philpot, 1975; Philpot, 1984). Parenteral treatment was chosen, and again the aim was to combat both *S. agalactiae* and *S. aureus* infections if possible. As the calving dates were unknown for many of the cows, intramammary DCT could not be used because the long milk withdrawal period of those drugs might have resulted in drug persistence after calving, thus requiring milk to be discarded for an extended period in early lactation. Parenteral amoxycillin combined with clavulanic acid was the treatment chosen for the dry cows.

3.2.3. Implementation

A total herd 'blitz' regime was chosen rather than partial 'blitz therapy', mainly due to the widespread nature of the infection in this herd and because this routine was simpler for the farmer to follow.

The milking cows, including heifers, and the dry cows were given a three day course of treatment. The treatments were administered immediately after the morning milkings. The teats were prepared as for milk sampling (Chapter 2.4.2.3.) prior to infusion of the intramammary antibiotics, and after the infusions, post-milking teat disinfection was undertaken.

The lactating cows were given procaine penicillin (Depocillin, Intervet UK Limited, Cambridge) by intramuscular injection, and nafcillin, dihydrostreptomycin and sodium benzylpenicillin (Nafpenzal MC, Intervet UK Limited, Cambridge) by intramammary infusion once daily for three days.

The dry cows were given amoxycillin and clavulanic acid (Synulox, Pfizer Ltd, Sandwich) by intramuscular injection once daily for three days.

The antibiotics were administered by veterinary researchers and students, as it was felt that the farmer could not be relied on to identify the cows correctly and administer the appropriate treatment.

Following treatment on the third day, the byre was disinfected and the liners of every cluster were discarded and replaced with new, unused liners. The old liners were removed from the farm for disposal. The cubicles were cleaned and fresh sawdust put down. The farmer was instructed to maintain the herd as a closed herd, and only to buy uncalved heifers as replacements if suitable homebred animals were unavailable.

3.2.4. Logistical considerations

Milk was discarded for the recommended statutory withdrawal period for the drugs used. The milk purchasing company were informed of the treatment, and undertook to check the bulk milk prior to collection after this period in order to ensure that no antibiotic residues were present in the milk. The farmer was instructed to withhold the milk, from the bulk tank, from any dry cow calving within a week after the 'blitz therapy', for one further week after the usual withdrawal period.

3.2.5. Costings

To calculate the cost of the 'blitz therapy', the loss in income due to discarding milk during the treatment period had to be considered along with the cost of the antibiotics. No costs were calculated for the visits or professional time.

Inspection of data from National Mastitis Council (Anon, 1998) showed that the average cow produces approximately 5,790 litres of milk per lactation. However, it was considered unlikely that the cows in the study herd would be achieving an average daily yield of 19 litres. And after considering the milk returns (Appendix 8), and considering wastage and milk for calves, it was estimated that the average yield

per lactation of a cow in this herd was 2500-3000 litres. The herd was assumed to calve all year round with each cow having an average lactation of ten months, therefore, giving the daily yield per cow as 10 litres/day.

The average price paid for milk in 1997 by milk purchasing companies was 22 pence per litre (ppl) (Anon, 1998). However, prior to the 'blitz therapy' the high BMSCC resulted in the imposition of a penalty of three pence per litre of milk produced. The penalty was a standard penalty imposed by the milk purchasing company on milk with BMSCC > 500,000 cells/ml. Therefore, the farmer received 19 ppl rather than the average 22 ppl, prior to the 'blitz therapy'.

3.3. Results

3.3.1. 'Blitz therapy'

Forty three cows were lactating at the time of the 'blitz therapy' and were given treatment with procaine penicillin (Depocillin, Intervet UK Limited, Cambridge) by intramuscular injection and nafcillin, dihydrostreptomycin and sodium benzylpenicillin (Nafpenzal MC, Intervet UK Limited, Cambridge) intramammary tubes, once daily for three days. Twelve cows were dry at the time of the 'blitz therapy' and were given intramuscular amoxycillin combined with clavulanic acid (Synulox, Pfizer Ltd, Sandwich), once daily for three days. One cow calved on the third day of the treatments. This animal was given amoxycillin combined with clavulanic acid (Synulox, Pfizer Ltd, Sandwich) on the first day of the 'blitz' and was then noticed to be close to calving and was subsequently separated from the herd. Following calving, the cow was treated with the same drug regime as was administered to the lactating cows during the 'blitz'; the cow was returned to the herd after the statutory milk withdrawal period. A milk sample was collected and analysed for antibiotic residues to ensure that the milk was safe to enter the bulk tank. The treatments administered to each cow are detailed in Appendix 6.

3.3.2. Problems encountered during the treatment

The 'blitz therapy' began on 18th March 1997 and the replacement of the liners, the byre disinfection and the bedding of the cubicles occurred on 20th March 1997. The longest withdrawal period for the antibiotics was three and a half days, however, when parenteral and intramammary treatments are used in conjunction the standard seven day milk withdrawal should be applied as the intramammary tubes used are not licensed for use along with parenteral antibiotics. Therefore, the milk would be able to be sold for human consumption from the 25th March 1997. The milk company was notified about the herd treatment and were monitoring the bulk tank for antibiotic residues prior to collection. Antibiotic residues were present in the bulk tank milk on 24th March 1997, but on the 25th March 1997 the bulk tank milk was free from antibiotic residues and could be collected for sale as was planned. Therefore, the total milk yield from this herd was discarded for one week.

One cow calved within one week of the 'blitz therapy', calving on 23rd March 1997 and her milk was withheld for one further week in order to ensure that no antibiotic residues resulted from the combined use of parenteral antibiotics with DCT. However, there were no records detailing when, or if, this cow was ever given DCT.

3.3.3. Costings

There were 44 cows (including the recently calved cow) in milk at the time of the treatment, and milk was discarded for seven days. The total volume of milk discarded was estimated to be $10 \times 7 \times 44 = 3{,}078$ litres. With the penalties being accounted for, the farmer was receiving 19 ppl. The total cost of the discarded milk was therefore $3{,}078 \times 0.19 = £585$.

The cost of the treatment of the herd included only the costs of the drugs. The intramammary infusions for the lactating cows cost £602 for the three day period, with the parenteral treatment for these animals for the same period costing £134. The parenteral antibiotics administered to the dry cows for the three days cost £121. These figures were the cost price direct from the drug wholesalers and, as most veterinary practices have a 50% mark up charge on all drug sales (SPVS fee survey

1997), this gave an estimated total cost for the 'blitz therapy' of £1,388. The cost of antibiotics used for the 'blitz therapy' are detailed in Table 5.

The whole herd 'blitz therapy' cost the farmer £1973, this included the cost of the drugs and the loss in revenue due to the milk being discarded for one week.

Antibiotic	**Volume/quantity of antibiotic used per cow**	**No. of cows**	**Total quantity of antibiotic used**	**Cost of antibiotic**	**Total**
Nafpenzal	4 tubes	44	4 × 44 × 3 = 528 tubes	£22.80 for 20 tubes	£602
Depocillin	20 ml	44	20 × 44 × 3 = 2640 ml	£5.06 for 100 ml	£134
Synulox	25 ml	12	25 × 12 × 3 = 900 ml	£20.99 for 100 ml	£189
Total cost of 'blitz therapy'					£925
with 50% mark up					£1388

Table 5: Costs of the 'blitz therapy' treatments

3.4. Discussion

The whole herd 'blitz therapy' regime was selected for this herd rather than the partial herd approach because the *S. agalactiae* infection was chronic and widespread: 77% of the herd were lactating at the time when treatment was being considered, and it was thought that the farmer could not be relied on to follow the treatment protocol necessary for the partial herd treatment. The high prevalence of *S. aureus* infection in the herd meant that antibiotics were chosen for the 'blitz therapy' which had a good activity against *S. agalactiae* and *S. aureus*. Nafcillin was selected as the intramammary treatment for the lactating cows because of its action against *S. aureus* (McKellar, 1991) and penicillin was chosen as the parenteral treatment for its known efficacy against *S. agalactiae* (Sandholm and others, 1990; McKellar, 1991). The treatment for the dry cows was chosen with the intention that it should eliminate *S. agalactiae* and reduce *S. aureus*, therefore, parenteral amoxycillin combined with clavulanic acid was chosen.

Whole herd 'blitz therapy' was carried out on the 56 cows in the herd, 43 were lactating, one was recently calved and 12 were dry at the time of the 'blitz therapy'. Unfortunately DCT could not be carried out in this herd because expected calving dates were unknown and, as a result, safe milk withdrawal periods could not be calculated. The direct cost of 'blitz therapy' was £1,973, labour costs were not included.

It was planned to monitor the herd closely for one year in order to check that infection did not recur. The procedure adopted and results of monitoring the herd are presented and discussed in Chapter 4.

Chapter 4

Monitoring

4.1. Introduction

Somatic cell counts may be used to estimate the degree of inflammation within the udder of a cow and hence the presence of mastitis (Dohoo and Meek, 1982; Booth, 1985; Sears and others, 1993; Cullor and Tyler, 1996). Therefore, BMSCC will provide a crude indication of the mastitis status of a herd (Westgarth, 1975; Booth, 1996; Logue, 1997). Consequently, it is the BMSCC, along with the TBC of a sample of bulk milk that is the basis of the legislation governing the quality of milk at the farm gate level. The European Community Health and Hygiene Directive 92/46 requires bulk milk to have a SCC < 400,000 cells/ml and a TBC < 100,000 cells/ml (Chapter 1.3.).

Since BMSCC acts as an indicator for the level of subclinical mastitis in a herd it is a cheap, convenient method for monitoring the response to any control measures or management changes and for monitoring the mastitis status of a herd. BMSCC are measured weekly by the milk company and payments are made based on three month geometric means. Milk companies impose price penalties for high BMSCC (Chapter 2.3.). Measurement of somatic cells are performed in the UK by automated Fossomatic counters (Blowey and Edmondson, 1995).

Another method used for monitoring the herd status is bulk milk bacteriology, which is a simple, specific, but insensitive method of monitoring the herd for the presence of bacteria in the milk (Godkin and Leslie, 1991; Lawrence, 1997) and is useful as a post-treatment check (Biggs, 1996). However, both these techniques, BMSCC and bacteriological examination of bulk milk, are more accurate for monitoring contagious pathogens as these tend to be shed in higher numbers than environmental

pathogens (Neave, 1975).

Somatic cell counts and bacteriological sampling at the individual cow and individual quarter level are more precise methods than at the bulk milk level for detecting the disease status of individual cows. Furthermore, data from quarter samples will provide the most accurate assessment of the prevalence of infection in the herd, as individual quarter SCC will not be affected by the dilution of low SCC milk from non-infected quarters of the same cow in an ICSCC or of all cows in a BMSCC (Reneau, 1986). However, the increased number of samples required for monitoring by such means increases the financial and time costs involved.

4.2. Materials and methods

In order to monitor the response to whole herd 'blitz' treatment in the study herd, a number of sampling techniques were used.

4.2.1. Bulk tank screening

At the bulk tank level, samples were collected for bacteriological examination weekly for the first 12 weeks after the blitz treatment, and monthly thereafter for nine months. Bulk milk SCC were monitored weekly for one year after the 'blitz' treatment. The milk company kindly forwarded the BMSCC to the veterinary school every week.

4.2.2. Herd screening

Herd screening was also performed to monitor the SCC and bacteriological status of the individual lactating cows. This also provided the ideal opportunity to monitor the *S. aureus* status of the herd. Initially, it was decided to carry out the herd screenings one month 'post-blitz' and then six months later to monitor the possible return of *S. agalactiae* infection. Two additional herd screenings were performed to follow the progression of the *S. aureus* infected cows. Therefore, for the November and January herd screenings, cows which were infected with *S. aureus* were targeted for further sampling.

Two 10 ml milk samples were collected from each quarter using sterile technique (Chapter 2.4.2.3.) from all the lactating cows on two different occasions, and from cows which were found to have *S. aureus* infection on two further occasions, over a one year period after the 'blitz' treatment. On each occasion, one sample was submitted for SCC and one for bacteriological culture.

Composite samples, made up of equal volumes of milk from all four quarters of one cow, were collected for bacteriological examination at the October herd screening due to technical difficulties. This reduced the accuracy of the herd screening on this one particular occasion, with the possibility of bacterial overgrowth from one quarter obscuring the presence of some pathogens from other quarters, and thereby an infected cow may have gone undetected.

4.2.3. Costings

4.2.3.1. Cost of monitoring

Costs involved in monitoring the herd for *S. agalactiae* and *S. aureus* infection included the cost of bacteriological examination of weekly, then monthly, bulk milk samples; the cost of bacteriological examination of individual quarter or individual cow milk samples; and the cost of IQSCC of all lactating cows during the herd screenings. The cost of somatic cell counting is 50 pence per sample, therefore, it costs £2 per cow.

4.2.3.2. Effects of somatic cell counts on production

Assuming an average reduction of 10% in milk yield for the cows in this study during the period when the BMSCC were increased above the EC standard, and assuming an average daily milk yield of 10 litres, a high SCC cow in this herd would produce 19.8 litres/day. The farmer could potentially lose an average of 22p daily for each cow with a high SCC, and calculated on a herd basis lose £11 each day if the cows had an increased SCC (calculations assume a ten month lactation, an even year round calving pattern, therefore meaning that approximately of 50 cows will be in milk at any one time, and a milk price of 22 ppl, with a three ppl deduction for high

BMSCCs).

4.3. Results

4.3.1. Bulk tank screening

4.3.1.1. Bacteriology

Over the one year period of monitoring the herd after 'blitz therapy' there was no evidence of *S. agalactiae* infection from the bulk milk samples at any time. However, some species of bacteria were detected in most of the milk samples collected, although in most cases, the bacteria isolated were considered to be non-pathogenic. *Staphylococcus aureus* was identified in 10 of the 22 bulk milk samples collected though these 10 samples were not consecutive samples. *Staphylococcus aureus* was consistently isolated from the last four bulk milk samples. Table 6 summarises the bacteriology results from the bulk milk samples.

	Month sample taken	**Date**	**Period following 'blitz therapy'**	**Bacteria isolated**
1	Mar-97	19	2 days	none
2		27	1 week	*Staphylococcus aureus*
3	Apr-97	4	2 weeks	*Staphylococcus aureus, Gemella morillorum, Staphylococcus* spp. and *Bacillus* spp.
4		17	3 weeks	none
5		23	4 weeks	*Staphylococcus aureus*
6	May-97	1	5 weeks	*Aeromonas* sp.
7		7	6 weeks	none
8		13	7 weeks	*Staphylococcus epidermidis*

Table 6: Bacteriology results from weekly and monthly bulk milk samples taken during the one year monitoring period following 'blitz therapy'.

9	May-97	21	8 weeks	*Staphylococcus scuiri*
10		29	9 weeks	*Pseudomonas cepacia*
11	Jun-97	4	10 weeks	*Staphylococcus aureus*
12		13	11 weeks	*Streptococcus* spp.
13		21	12 weeks	none
14	Jul-97	18	4 months	*Staphylococcus aureus*
15	Aug-97	18	5 months	*Staphylococcus* spp.
16	Sept-97	19	6 months	none
17	Oct-97	16	7 months	*Staphylococcus aureus*
18	Nov-97	19	8 months	*Staphylococcus epidermidis* and *Bacillus* spp.
19	Dec-97	17	9 months	*Staphylococcus aureus* and *Bacillus* spp.
20	Jan-98	14	10 months	*Staphylococcus aureus* and *Streptococcus* spp.
21	Feb-98	17	11 months	*Staphylococcus aureus, Streptococcus* spp. and *Bacillus* spp.
22	Mar-98	17	12 months	*Staphylococcus aureus*

Table 6 (cont.): Bacteriology results from weekly and monthly bulk milk samples taken during the one year monitoring period following 'blitz therapy'.

4.3.1.2. Somatic cell counting

The weekly BMSCCs were within the EC directive 92/46 standard limits (< 400,000 cells/ml) except on three weekly occasions, once each in July 1997, February 1998 and March 1998 (Table 7). However, the monthly BMSCC, based on the three month geometric mean, for one year after the 'blitz therapy' were all less than 400,000 cells/ml. Figure 6 demonstrates the trend in BMSCC over the one year monitoring period and compares it with the two years prior to the investigations.

	3 month geometric average BMSCC ($\times 10^3$)	BMSCC ($\times 10^3$) 1st week	BMSCC ($\times 10^3$) 2nd week	BMSCC ($\times 10^3$) 3rd week	BMSCC ($\times 10^3$) 4th week
Mar-97	502	493	398	-	297
Apr-97	365	284	281	286	260
May-97	281	250	193	244	211
Jun-97	245	214	222	255	258
Jul-97	242	310	**435**	209	186
Aug-97	232	232	220	135	213
Sept-97	209	301	160	124	155
Oct-97	162	126	148	148	90
Nov-97	155	146	275	134	153
Dec-97	165	259	154	159	306
Jan-98	184	253	135	243	-
Feb-98	250	**589**	232	347	384
Mar-98	323	309	360	389	**611**
Apr-98	352	214	360	311	316

Table 7: Weekly bulk milk somatic cell counts (BMSCC) for the quota year 1997-1998.
BMSCC over the maximum acceptable standard given by the EC directive 92/46 of 400,000 cells/ml are shown in bold print.

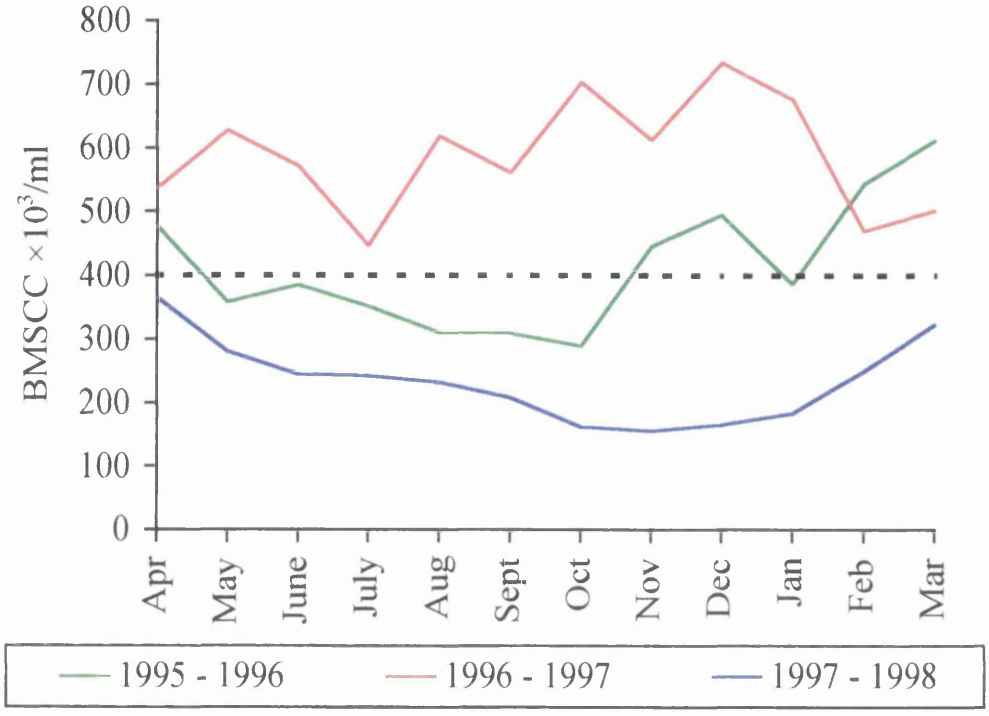


Figure 6: Bulk milk somatic cell counts (BMSCC) for two years prior to the 'blitz therapy', and the one year monitoring period following it.
----- denotes the maximum BMSCC acceptable under the EC Health and Hygiene directive 92/46.

A decrease in BMSCC was seen after the first farm visit in December 1996. The milking routine improvements and the 'Five Point Plan' were implemented in February 1997 and the 'blitz therapy' occurred in March 1997. From Figure 6 it can be seen that the BMSCC showed an immediate reduction. This decrease continued until November 1997 at which time there was the lowest BMSCC. This level was maintained until December 1997 after which a steady increase was seen. However, the BMSCC was maintained below 400,000 cells/ml for one year after the 'blitz therapy'.

4.3.2. Herd screening

4.3.2.1. Bacteriology

All cows which were in milk at the time of the first two herd screenings were sampled to monitor for any reoccurrence of *S. agalactiae* infection, and cows were selected to monitor the prevalence of *S. aureus*, in addition to the presence of *S. agalactiae* infection, at the next two visits. Herd sampling was undertaken in April 1997 (one month after the 'blitz therapy'), October 1997 (seven months after the 'blitz therapy'), November 1997 (eight months after the 'blitz therapy') and January 1998 (ten months after the 'blitz therapy').

Streptococcus agalactiae infection was identified in only one quarter of one cow during the April 1997 herd sampling. This cow was dry at the time of the 'blitz therapy' and calved on the 23rd March 1997 when her milk was withheld from the bulk tank for an extra week as she had calved within a week of 'blitz therapy'. After isolation of *S. agalactiae* in the April 1997 herd screening, this cow was subsequently treated using the regime for lactating cows during the 'blitz therapy', i.e. treated with nafcillin intramammary infusions and parenteral penicillin once daily for three days. The cow was separated from the herd and milked using a different milking machine. Following treatment, the cow was re-sampled prior to re-joining the herd. On repeat sampling the cow was found to be free from *S. agalactiae* infection. The other cows which were dry at the time of the 'blitz therapy' were sampled to ensure that they had no remaining *S. agalactiae* infection. Bacteriological culture of milk samples from these cows failed to detect any infection.

There was no further evidence of *S. agalactiae* infection in any cows from any of the herd samples. However, *S. aureus* was identified from several cows and often on repeated occasions. Table 8 demonstrates the cows from which *S. aureus* was identified and when it was identified.

Cow ID	Dec-96/ Jan-97	Apr-97	Oct-97	Nov-97	Jan-98
10	+		+		+
16	+		+		+
28				+	
29	+	+			
35	+		+		
69	+				
71		+	+		
204	+				
3P		+			+
A4	+				
A6			+		+
A8		+	+		
A10					+
B5	+	+			
B8				+	
C10				+	+
D1			+		
D5				+	+
D8	+				
D10					+
F3				+	
F6				+	+
F9					+
F10					+

Table 8: Cows from which *S. aureus* was identified, and the month(s) in which it was identified.
Cow identification as detailed in Appendix 6.

An example of the full results as recorded in the database for an individual cow are presented in tabular form in Appendix 1.

4.3.2.2. Individual cow somatic cell counting

The first two ICSCC samples which were taken during the herd screening were compared to the ICSCC taken in November 1996 prior to intervention using the one way ANOVA test followed by a Newman-Keuls multiple range test (Chapter 2.4.4.1.1.) to identify at which times there were significant differences in ICSCC. ICSCC were compared between times one (pre-intervention), two and three (post-intervention). Cow was entered into the model as a random effect to control for variation attributable to individual animals. The post-intervention ICSCC at both

post-intervention screenings were significantly lower than pre-intervention ($p=0.001$).

4.3.3. Costings

4.3.3.1. Cost of monitoring

In total, there were 22 bulk milk samples collected for bacteriological examination, costing £220 over the one year herd monitoring period. The bacteriological examination of the individual quarter or individual cow samples taken during the four herd screenings cost a total of £3,870, and the IQSCC cost £246 for the four herd screenings. Therefore, the total cost of the herd monitoring for one year after the 'blitz therapy' was £4,336. The total costs are itemised in Table 9.

Analysis	Type of sample	No. of samples	Cost of analysis	Calculation	Total
Bacteriology	Bulk tank samples	22	£10 per sample	10 × 22	£220
Bacteriology	Herd screenings on four occasions	387	£10 per sample	10 × 387	£3870
Somatic cell counting	Herd screenings on four occasions	492	50 pence per sample	0.5 × 492	£246
TOTAL COST					£4336

Table 9: Costs involved in monitoring the herd after the 'blitz therapy'.

4.3.3.2. Effects of somatic cell counts on production

Due to the fact that the BMSCC remained below 400,000 cells/ml for one year after the 'blitz therapy' there was no penalties imposed, and it was assumed that there was no reduction in milk yield. Therefore, the farmer should have been able to earn £11 more each day, giving a potential increase in income of £4,015 per year.

4.4. Discussion

It can be seen from the bulk milk samples that the BMSCC was maintained below the EC standard of 400,000 cells/ml for at least one year after whole herd 'blitz

therapy' was performed in this herd (Figure 4). *Streptococcus agalactiae* infections were successfully eliminated from this herd, despite the isolation of this bacteria from one cow following the 'blitz therapy'. However, there did not appear to be any beneficial effect on the S. *aureus* infections.

The bacteriologists reported that CAMP testing was carried out on any *Streptococcus* spp. isolated from this farm. This identification process is not standard procedure, but was performed in order to be confident that *S. agalactiae* had not re-appeared in this herd.

The isolation of *S. agalactiae* infection from a single cow following treatment suggested that the amoxycillin combined with clavulanic acid (Synulox, Pfizer Ltd, Sandwich) regime in the dry period may have been an inadequate treatment for *S. agalactiae* infection. The occurrence of infection in this cow highlights how important it is to pay careful attention to the dry cows when undertaking 'blitz therapy'. However, the cow may have become infected in the period after calving and prior to the herd screening. The infection in this cow could potentially have spread to the rest of the herd resulting in failure of the 'blitz therapy' approach. The improvements made to the milking routine may have played an important part in restricting the spread of the *S. agalactiae* infection from this cow to the others in the herd. Following elimination of the bacteria from this cow, the other dry cows were sampled after calving to verify that they were free of infection prior to joining the milking herd. All other cows which were treated during their dry period were found to be free of *S. agalactiae* infection after calving.

The cure rate for *S. agalactiae* infection in this study was 98.2%. High cure rates have been achieved by others, using various other intramammary or parenteral antibiotics. Moss and others (1998) reported a 98.7% cure rate using a three day course of injectable penethamate hydroiodide, no intramammary preparations were used. Roberts and others (1963) achieved 90% eradication using intramammary penicillin. Weaver and others (1986) recorded a 90% recovery rate using a one or two day course of intramammary procaine penicillin G, with and without,

novobiocin. Bushnell (1980) reported expected cure rates of > 90% using a single infusion of intramammary procaine penicillin G. Procaine penicillin G cured 89.8% of cows with *S. agalactiae* infection in a study by Deneke and others (1993). Erskine and Eberhart (1990) reported that *S. agalactiae* infections were eliminated using intramammary infusions of a procaine penicillin and novobiocin preparation from 88.6% of treated cows. Zecconi and others (1993) achieved a 88.2% cure rate in lactating cows, using penethamate hydroiodide/dihydrostreptomycin preparation, and a 90% cure rate in dry cows. Johnston (1975) reported the eradication of *S. agalactiae* from three herds using erythromycin intramammary infusions, the initial cure rate was 77% with some more responding to repeat treatments. Cefoperazone treatment of *S. agalactiae* infected quarters was reported by Bertocchi (1991) to reduce infection in four herds to zero in six to fourteen months.

The outlay for the investigations (Chapter 2), treatments (Chapter 3) and monitoring in this herd totaled £6,890, while the estimated gain in milk yield for the year was worth £4,015. Therefore, it woul take twenty and a half months to pay for the intrevention on this farm. The calculations were simplified by being based on the lack of penalties following successful reduction in BMSCC, at an average milk price of 22 ppl for 1997.

Comparison of the costs for implementation of 'blitz therapy', and for monitoring the herd, shows that the monitoring exercise was the more expensive element (£4,336 versus £2,708). However, due to the poor management of this herd it was decided to monitor the herd closely in order to provide a continual incentive to the farmer to improve and maintain the hygiene of the milking routine. Unfortunately, the record keeping remained poor, and mastitis cases and treatment administered were still not recorded adequately, even though it was a legal requirement to record medicine useage on farms. However, calving dates and service dates were recorded.

The repeated isolation of *S. aureus* from bulk milk samples, especially over the last four months of the monitoring exercise, where *S. aureus* was isolated from consecutive samples, and the increase in the BMSCC over this period suggested that

infection with *S. aureus* was not controlled adequately by the total herd 'blitz therapy', and/or improvements in the milking routine. Measures to reduce the *S. aureus* infection were planned and implemented and these are discussed in Chapter 5.

Chapter 5

Attempted reduction of *Staphylococcus aureus* infection in high cell count cows

5.1. Introduction

Staphylococcus aureus is a contagious mastitis pathogen which most often causes chronic subclinical infections with clinical cases occurring only occasionally (Cullor and Tyler, 1996). *Staphylococcus aureus* is difficult to eliminate from a herd due to: (1) its ability to survive for extended periods on the skin (McDonald, 1977), (2) its ability to survive within neutrophils (Craven and Anderson, 1979; Madgwick and others, 1989; Sandholm and others, 1990; Bramley, 1992) and, (3) poor bacteriological cure following antibiotic treatment (Ziv, 1980; Bramley and Dodd, 1984; Fox and Gay, 1993). There are different strains of *S. aureus* which have varying virulence and some produce β-lactamase which makes them resistant to penicillins (Sandholm and others, 1990; McKellar, 1991; Fox and Gay, 1993; Vestweber and Leipold, 1993). The 'Five Point Plan' is an important control measure for contagious mastitis and can bring about a reduction in the spread of *S. aureus* (Smith and Hogan, 1996), but for eradication purposes, segregation and culling of individual cows may be necessary (Wilson, 1980; David, 1993).

Initial milk samples collected from a pre-selected sample of the herd in December 1996 showed that, 42.9% (9/21) of cows had *S. aureus* infection in one or more quarters (Chapter 2.5.2.3 and Chapter 2.5.3.). *Staphylococcus aureus* was isolated from 10 of the 22 bulk milk samples taken during the one year monitoring period (Chapter 4.3.1.1.). Results of bacteriological examinations of samples from the last four herd screenings showed that 27.9% (24/86) of cows had *S. aureus* infections, and, of these *S. aureus* infection had been detected in eight cows on more than one

occasion (Table 8).

Whole herd 'blitz therapy' was aimed primarily at eliminating *S. agalactiae* from this herd (Chapter 3). The response to the treatment was monitored closely over the following year by sampling from the bulk tank and herd screening (Chapter 4). On identifying the high prevalence of *S. aureus* infection in the herd and the gradual rise in BMSCC towards the end of the one year monitoring period, it was decided to implement further control measures in an attempt to reduce and control infections with *S. aureus*. For this to occur the organism had to be eliminated from individual chronically affected cows.

5.2. Materials and methods

5.2.1. Cow selection

Six cows were chosen, based on repeated bacteriological samples, for treatment. Cows in which *S. aureus* infection was detected, especially in more than one milk sample, and which were lactating at the time of the proposed trial treatment were selected. Three cows in their first lactation and three cows in later lactations were selected for therapy. The cows used in this study were three to seven years old and their ICSCC varied from 11,000 to 7,010,000 cells/ml. Their identity and bacteriological history are shown in Table 10. Appendix 7 summarises the bacteriology results for all the cows in the herd, highlighting the cows from which *S. aureus* was isolated. Table 11 summarises the ages, the ICSCCs and the bacteriological information for the six cows which were selected for treatment to attempt to reduce *S. aureus* infection.

Cow ID	Isolation of *Staphylococcus aureus*																
	Dec-96/Jan-97				Apr-97				Oct-97	Nov-97				Jan-98			
	lf	lh	rf	rh	lf	lh	rf	rh	comp	lf	lh	rf	rh	lf	lh	rf	rh
10	-	+	-	+	n	n	n	n	+	n	n	n	n	-	+	-	-
A6	n	n	n	-	n	n	n	n	+	n	n	n	n	+	n	n	+
D5	n	-	n	n	n	-	n	-	n	-	+	-	+	-	+	-	-
F3	n	n	n	n	n	n	n	n	n	+	+	-	+	-	-	n	-
F6	n	n	n	n	n	n	n	n	n	-	+	-	+	-	+	-	-
F10	n	n	n	n	n	n	n	n	n	n	n	n	n	-	+	-	+

Table 10: Bacteriology results of cows which were selected for treatment, indicating when and in which quarters *Staphylococcus aureus* was isolated.
+ indicates that *Staphylococcus aureus* was isolated. - indicates that *Staphylococcus aureus* was not isolated. n indicates that a sample was not collected for examination. comp. - composite milk sample. lf - left front quarter. lh - left hind quarter. rf - right front quarter. rh - right hind quarter.

Cow ID	Age (years)	ICSCC × 10^3/ml (Nov. 1996)	ICSCC × 10^3/ml (April 1997)	ICSCC × 10^3/ml (Oct.1997)	ICSCC × 10^3/ml (Nov.1997)	ICSCC × 10^3/ml (Jan. 1997)	No. of quarters with *S.aureus*	No. of occasions *S.aureus* isolated
10	7	613	n	128	854	292	2	3
A6	7	783	n	180	1141	7010	2	2
D5	4	409	266	n	16	271	2	2
F3	3	n	n	n	5	13	3	1
F6	3	n	n	n	65	2478	2	2
F10	3	n	n	n	n	11	2	1

Table 11: Summary of individual cow somatic cell counts (ICSCC) and bacteriological information collected, prior to treatment, for the six cows which were selected for treatment.
n indicates that a sample was not collected for examination

5.2.1.1. Antibiotic treatment

The main criterion for selection of antibiotics was efficacy against *S. aureus*. Secondary, but important, considerations were milk withdrawal periods and cost of the antibiotic. Ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby), a β-lactamase resistant, broad spectrum, bactericidal cephalosporin antibiotic preparation for parenteral administration was selected (Prescott and Baggot, 1993; Anon, 1998). Ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby) was selected for its excellent efficacy against gram-positive bacteria and because of the nil milk withdrawal period for this drug. Treatment was given for a prolonged period, ten days instead of the recommended standard three to five days, in order to increase the chance of clearance of the *S. aureus* infection from the mammary gland of the cows.

5.2.1.2. Treatment protocol

The six cows selected for treatment against *S. aureus* infection were injected once daily for ten days. Ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby) was administered by intramuscular injection once daily for 10 days at a dose rate of 1 mg/kg by veterinary personnel every morning following milking. The cows averaged 500 kg, therefore were given a daily dosage of 50mg.

5.2.2. Milk samples

5.2.2.1. Bacteriology

In order to monitor the response to ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby) therapy, milk samples were collected starting four days after the end of the ten day treatment period and bacteriological culture was performed on all samples. Composite milk samples were collected from individual cows using a sterile sampling technique (Chapter 2.4.2.3.), prior to milking in the morning. Composite milk samples and bulk milk samples were collected at the start of the treatment and then collected on a weekly basis for six weeks after the ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) treatment.

5.2.3. Costings

Costs were obtained for the ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) used and for the sterile water (Water for Injection, Animalcare, York), which was used to suspend the antibiotic. Estimated costs for labour were not included. Also included in the costing was the cost of bacteriological examinations of the milk samples collected before and after the treatment to monitor the response to treatment. The standard charge of £10 per sample was made. A 4g vial of ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) cost £24.30 and 100 ml of sterile water (Water for Injection, Animalcare, York) cost £1.80. To reconstitute the powder 80 ml of sterile water were required and this worked out that 1 ml essentially provided 50 mg of ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby). Therefore each cow required 10 ml of the reconstituted drug. The cost of a bottle of the reconstituted drug was £25.74 and every cow needed 1.25 bottles, working out at a cost of £32.20 per cow for the ten day treatment.

5.3. Results

5.3.1. Treatment

The six selected cows were all given daily intramuscular injections of 1 mg/kg ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) for ten consecutive days. Treatment was initiated on the 10th March 1998.

5.3.2. Bacteriology

The bacteriology results, for milk samples from the six cows and the bulk tank, are given in Table 12. *Staphylococcus aureus* infection was detected in five of the six cows after the ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) treatment was administered. However, the number of *S. aureus* colonies detected on culture of the milk samples was reduced in comparison to the pre-treatment samples, only one or two colonies were recorded in the follow up samples instead of the profuse growths which were detected prior to treatment. One first lactation cow (F3) was found to have no *S. aureus* in any of the

six weekly samples. The other two first lactation cows (F6 and F10) were positive on five of the six occasions. *Staphylococcus aureus* was isolated from all six bulk milk samples.

Week no.	Date	Bulk milk	Cow identification					
			10	A6	D5	F3	F6	F10
1	10/3/97	+	+	+	+	-	+	+
2	17/3/97	+	+	+	+	-	-	+
3	25/3/97	+	+	+	+	-	+	-
4	1/4/97	+	+	DRY	+	-	+	+
5	8/4/97	+	DRY	DRY	DRY	-	+	+
6	16/4/97	+	DRY	DRY	DRY	-	+	+

Table 12: Isolation of *Staphylococcus aureus* in six cows following treatment with ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby).
+ indicate that *Staphylococcus aureus* was isolated. - indicates that *Staphylococcus aureus* was not isolated

5.3.3. Costings

In total there were 35 milk samples submitted for bacteriological examination. Therefore, the monitoring cost £350.

Ceftiofur administered at a dose rate of 1 mg/kg for the six cows for the treatment period of ten days resulted in a total of 500 mg/cow for each of the six cows for the treatment period, i.e. 7.5 vials of ceftiofur and 640 ml sterile water (Water for Injection, Animalcare, York). The total cost of the treatment was £194 and with a 50% mark up the cost of the drugs was £291. Therefore, the total cost involved in attempting to reduce the S. *aureus* infection in six clinical carriers in this herd was £641.

5.4. Problems encountered during the treatment regime

Three of the cows selected for treatment could not be sampled for the full six weeks. One of these cows was sampled for three weeks only and the other two were sampled for four weeks. Of the three cows which were not sampled for the entire six weeks, two (10 and D5) were not

sampled for the agreed six weeks because they were dried off due to low milk yield. These cows were not due to calve for a further three and five months respectively, but had been lactating for 10.5 and 4.5 months respectively. These low yielding cows were dried off and given DCT because their milk would have increased the BMSCC had it entered the bulk tank. The third cow (A6) was due to calve in one month and, therefore, was dried off and given DCT. The three cows which were sampled for the full six weeks were the first lactation cows. One cow (F3) had no *S. aureus* isolated during the six week sampling period. However, the last milk sample collected prior to the ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) treatment was also negative for *S. aureus* infection, in spite of *S. aureus* being present in three quarters on one occasion five months before this treatment was attempted.

5.5. Discussion

At the time of treatment, it was hypothesised that the three first lactation cows were likely to respond better than the three older cows, because in younger cows, infections were likely to be new and the cows' immune system was likely to respond more effectively (McKellar, personal communication). Infections in older cows tend to have a longer duration and cause more extensive tissue damage than in younger cows (Reneau, 1986).

The results of bacteriological examinations showed that *S. aureus* was not eliminated from any of the six cows treated with ceftiofur. In five cows infection was continued and in one cow (F3) infection was likely to have been eliminated prior to treatment with ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby). However, the level of infection was considered to be reduced as there were only one or two colonies cultured on the agar plates following the antibiotic treatment. The first lactation cow (F3) from which *S. aureus* infection was not detected at any point during this study may have cleared the infection spontaneously prior to the ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited, Corby) treatment or alternatively the cow may have only been shedding *S. aureus* intermittently. This reduction in the level of infection should have, in the short term,

increased the cows' milk yields and reduced the ICSCC, unfortunately due to farm circumstances neither the cows' milk yield or ICSCC were monitored.

The parenteral penicillin and the intramammary nafcillin which were used for the 'blitz therapy' (Chapter 3.2.2) appeared to have a minimal affect on the *S. aureus* infections therefore a different approach was required. It was decided to attempt reduction of the *S. aureus* infections in this herd using a parenteral treatment for a prolonged period. Treatment was administered for a prolonged period (ten days) in order to exceed the lifespan of neutrophils thereby allowing the antibiotic to be effective against intracellular bacteria (Belschner and Sears, 1998; McKellar, personal communication). Neutrophils survive in the circulation for 7-14 hours and in the tissue for 2-3 days, giving a total lifespan of 3-4 days (Jain, 1993). Belschner and Sears (1998) found that an extended treatment period of ten days did improve the clinical cure rate of chronic *S. aureus* infection. Ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby) was chosen due to its known effacacy against gram-positive bacteria and nil milk withdrawal period. It was hoped that the systemic route would allow better distribution within the udder tissue. Given that the selected cows had high SCCs and had been identified as *S. aureus* infected there would have been a degree of inflammation in the udder and it was thought that the cephalosporin would be able to cross into the milk. However, after the six week period when it was realised that the treatment had been unsuccessful, milk samples, which had been taken during the treatment period and stored, were checked for the presence of any inhibitory products. To investigate for the presence of any inhibitory products milk samples taken after the initiation of treatment were cultured along with *S. aureus* and another susceptible *Staphylococcus aureus* (1964 Compton strain), if there were inhibitory products present the expectation was that these bacteria would be unable to grow. From this test procedure, there was no evidence of any inhibitory products in the milk samples, thereby explaining, in part, the failure of the parenteral ceftiofur (Excenel Sterile Powder, Pharmacia and Upjohn Limited Limited, Corby) to reduce the *S. aureus* infection.

The cure rate of *S. aureus* intramammary infections can be influenced by localisation of infection within the quarter, the age of the cow, persistence of shedding of *S. aureus* before drying off, the SCC of infected quarters, and the number of quarters infected with *S. aureus* per cow (Sol and others, 1994). The probability of eliminating *S. aureus* infection decreases with increasing SCC and age (Sol and others, 1994). The possible cure rate in this study was one out of six cows (16%), and this was probably due to self cure prior to ceftiofur treatment as the sample taken immediately before treatment was found to be free of *S. aureus*. However, this figure cannot be extrapolated to other herds because of the small number of animals involved. It can be concluded, in agreement with previous work that attempts to eliminate *S. aureus* using antibiotic treatment during lactation is unlikely to be successful. In this study and in work by Soback and others (1989) it was found that parenteral ceftiofur had no activity in milk and would not be an antibiotic of choice for the treatment of high SCC cows by the systemic route.

5.6. Further work

Further control measures could include changing the antibiotic preparation used for routine DCT to a preparation with better activity against *S. aureus* and longer persistence . It was shown by Marco and others (1996) that cloxacillin (Orbenin Extra DC, Pfizer Ltd, Sandwich) used at drying off resulted in greater cure rates than cephalonium (Cepravin dry cow, Schering-Plough Animal Health, Uxbridge) and cloxacillin and ampicillin (Kloxerate Plus, Fort Dodge Animal Health, Southampton), and had a good action against *S. aureus* infections, with cure rates of 72.7% as compared to 58.1% and 55.4% with the other preparations respectively.

Two cows (10 and D5) were given a three day course of parenteral tylosin (Tylan 200, Elanco Products Limited, Basingstoke) at drying off, accompanied by DCT using cloxacillin (Orbenin Extra DC, Pfizer Ltd, Sandwich). Tylosin (Tylan 200, Elanco Products Limited, Basingstoke) was chosen because of its good tissue distribution, high intracellular concentrations and activity against gram-positive organisms. However, this product is only

licensed for intramuscular use and has a long milk withdrawal period. Therefore the treatment was given parenterally at drying off. The cows will be sampled, weekly for six weeks, after calving to monitor for the presence of *S. aureus* infection. At the time of writing, these cows have yet to calve.

Chapter 6

General discussion

Mastitis is a very important disease with huge economic implications to the dairy industry (Janzen, 1970; Wilson and Kingwill, 1975; Booth, 1988; Sischo and others, 1990; Bramley, 1992; Esslemont and Peeler, 1993; Smith and Hogan, 1993; Muldoon, 1995; Rebhun, 1995). The adoption of the European Community Health and Hygiene Directive 92/46 a the farm gate in January 1998 forced farmers to pay greater attention to the control of mastitis in order to produce milk of a sufficient standard to meet market requirements. The crisis affecting the farming industry of the UK over recent months, in the aftermath of BSE has led to depressed incomes for dairy farmers. Decreasing milk and stock prices have meant that farmers need to be able to produce milk ever more efficiently, which in turn, necessitates excellent disease control in order to mitigate the negative effects of ill health on productivity.

Mastitis control depends largely on good herd management and milking hygiene, and strict adherence to the 'Five Point Plan' (Booth, 1975b; Jackson, 1980; Smith and Hogan, 1996). Herd recording is an essential part of determining and monitoring the prevalence of disease. The recording of clinical cases of mastitis may increase the farmer's awareness of the disease and thus lead to the identification of recurrent or persistent cases (Blowey, 1986). Prompt treatment of clinical cases and the removal of recurrently or persistently infected cows will reduce the risk of spread of infection to other cows. The possibility of breeding for increased resistance to mastitis infection is the subject of ongoing research (Hamori, 1980; Shook, 1993).

The sixty cow dairy herd which provided the basis for this study had a persistently increased BMSCC for quota years 1995/1996 and 1996/1997. The milk company purchasing the milk were threatening to terminate the contract with the farmer if there was no attempt to improve

this situation. It was subsequent to this warning that the farmer sought veterinary advice and investigation of milk production practices in this herd were initiated in November 1996. The investigation began by the examination of historical data from the herd, followed by a farm visit where the milking routine and the milking hygiene were observed and assessed. Milk samples were collected from cows selected on the basis of high ICSCC from the historical data and a positive CMT result. The milk samples were submitted for further investigations. Bacteriological examinations were carried out on the milk samples collected and it was found that 67% of the cows sampled were infected with *S. agalactiae* and 33% were infected with *S. aureus*. The buildings and cubicles were, in general, in a poor state of repair.

Immediate implementation of control measures were essential to prevent the farmer going out of business. Careful consideration of the historical information, and the results of a series of on-farm investigations, led to the implementation of control measures comprising the coordinated adoption of the 'Five Point Plan', in combination with whole herd 'blitz therapy' to eliminate infection with *S. agalactiae*. Further control measures involving improving the milking routine and farm records were also implemented: udders were washed using running water from a hose, separate paper towels were used to dry the udder of each cow, clusters were regularly dipped in disinfectant during the milking process, iodine based teat disinfectant was used as a post-milking teat dip after each milking, routine DCT was carried out and improvements were made to the cleaning of the milking line and data recording.

The whole herd 'blitz therapy' consisted of a combined regime of intramammary and parenteral antibiotics. The choice of antibiotics was made with the primary aim of eliminating *S. agalactiae* and the secondary aim to reduce *S. aureus*. This regime was chosen to ensure adequate tissue distribution and penetration by the antibiotics. It was considered to be the therapy most likely to achieve the combined study objectives, and was the only course of action likely to avoid the milk company refusing to purchase milk from this farm. The selection of antibiotics was influenced by the preparations available, by the desired route, the requirement for once daily administration because of reliance on veterinary administration, and the length of the milk withdrawal period. Concern about antibiotic residues in milk

resulted in intramammary DCT not being implemented. There were dry cow preparations available with short withdrawal periods of seven days which could have been used, but the farmer could not be relied upon to identify the cows correctly and antibiotic contamination of the bulk tank may have been the result. However, for this herd, parenteral treatment with amoxycillin combined with clavulanic acid (Synulox RTU, Pfizer Ltd, Sandwich) was used as the antibiotic in the 'blitz therapy' for the non-lactating cows. This antibiotic was selected as it is a broad spectrum antibiotic with resistance to β-lactamase producing *S. aureus*.

The identification of *S. agalactiae* in a single cow one month after the 'blitz therapy' caused concern about the efficacy of the chosen therapy. This cow was dry during the 'blitz therapy' and, therefore, received a three day course of amoxycillin combined with clavulanic acid (Synulox RTU, Pfizer Ltd, Sandwich). The cow calved three days after the last day of the 'blitz therapy' and, as a result, her milk was discarded for an additional week i.e. it was ten days after the 'blitz therapy' before her milk was allowed to be sold for human consumption. New infections are considered to be the most common and most logical explanation for apparent treatment failures (Sandholm and others, 1990). There are two possible theories to explain the re-occurrence of *S. agalactiae*: (1) the possibility that infection may have survived in the udder following the 'blitz therapy' or, (2) new infection may have occurred in the period between calving and the first herd screen, one month after the 'blitz therapy'. The other cows which were treated during their dry period were subsequently sampled after calving to ensure that they were not harbouring any *S. agalactiae* infection. Bacteriological examination of milk samples collected from these cows failed to detect *S. agalactiae*. Milk samples were collected after the second treatment to verify that the infection had been cleared from the individual cow from which *S. agalactiae* was isolated. *Streptococcus agalactiae* was not detected from this cow, or any other cows, or from the bulk tank, for the remainder of the one year monitoring period. The lack of transmission of this contagious pathogen prior to detection in the cow may suggest that the infection was a failure in treatment rather than a new infection.

It was concluded that the 'blitz therapy' was successful in eliminating *S. agalactiae* from this herd. The BMSCC was maintained below the EC standard for one year. The 98% cure rate achieved in this study was considered to be very good and was consistent with cure rates achieved for herd treatment of *S. agalactiae* infection in other studies, which ranged from 88.2% to 98.7% (Bushnell, 1980; Weaver and others, 1986; Erskine and Eberhart, 1990; Deneke and others, 1993; Zecconi and others, 1993; Moss and others, 1998). However, the BMSCC showed a slight increase in the latter part of the monitoring period and *S. aureus* was consistently isolated from bulk tank samples from February 1998. This apparent increase in *S. aureus* infection may have been due to the elimination of *S. agalactiae* infections, allowing S. *aureus* to become more of a problem, perhaps due to the phenomenon of the lack of inhibition by other major pathogens (Rainard and others, 1988; Biggs, 1996).

It was decided to attempt to reduce the prevalence of *S. aureus* by treating a number of the cows with antibiotics. Six cows were selected for initial treatment with a prolonged course of parenteral ceftiofur (Excenel sterile powder, Pharmacia Upjohn, Corby). The nil milk withdrawal period for this drug due to its high level of safety in humans, reflected by its high maximum residue limits, meant that milk need not be discarded from treated cows, thereby simplifying the management of the treated cows. However, the concentration of ceftiofur (Excenel sterile powder, Pharmacia Upjohn, Corby) in milk was assessed to ensure that sufficient concentrations of the drug did penetrate the udder. It was found that there was no evidence of any inhibitory products in the milk and therefore it was not surprising that ceftiofur (Excenel sterile powder, Pharmacia Upjohn, Corby) was not able to eliminate *S. aureus* infection in these cows. Soback and others (1989) when measuring the concentration of cetiofur in milk after intravenous or intrmuscular administration failed to find any ceftiofur in the milk. Wheeler (1998, personal communication) found that the minimum inhibitory concentration of ceftiofur against European isolates of *S. aureus* was 20 times more than the maximum concentration of ceftiofur which could diffuse into the udder after recommended dosing. However, in this study the numbers of *S. aureus* colonies were reduced which could suggest that some ceftiofur did penetrate the udder, but the cows could also have self-cured. The number of cows which were treated were too few to make any significant statements

about the value of this treatment, although it does suggest that it was not very successful for eliminating *S. aureus*. Watson (1998) suggested that using combined therapy of parenteral tylosin (Tylan 200, Elanco Products Limited, Basingstoke) and intramammary infusions with cloxacillin (Orbenin Extra DC, Pfizer Ltd, Sandwich) at drying off may be effective in reducing the rate of new infections and increasing the cure rate of existing infections caused by *S. aureus*. The results from tylosin (Tylan 200, Elanco Products Limited, Basingstoke) treatment of two cattle in this herd at drying off are awaited and no judgment can be made to date. Further work to find a successful antibiotic for the reduction of *S. aureus* infections is necessary with larger numbers of cows than used in this preliminary study. Biggs (1998) showed a favourable SCC response in seven cows with persisitent *S. aurues* infections using 10 mg/kg tilmicosin (Micotil, Elanco Products Limited, Basingstoke) at drying off.

The use of intramammary infusions carries with it a risk that bacteria may be accidentally introduced into the teat via the teat canal during the infusion process (Murphy and Stuart, 1954). Boyer (1997) and Edmondson (1997) both reported outbreaks of clinical mastitis following 'blitz therapy'. In both cases erythromycin intramammary preparations were used, the treatments were administered by the farmers, and the teats were prepared for infusion using a post-milking teat disinfection. Erythromycin is a narrow spectrum antibiotic, effective only against gram-positive bacteria and *Mycoplasma* spp.. The erythromycin treatments were repeated, for two or three consecutive milkings, which increases the risk of introducing bacteria into the teat canal. Biggs (1997) reported the use of surgical spirit to prepare teats prior to infusion of penicillin and streptomycin (Streptopen Milking Cow, Mallinckrodt Veterinary Ltd, Middlesex) and found no ensuing cases of clinical mastitis. Surgical spirit was used for teat preparation prior to intramammary infusion in the current study, and no cases of clinical mastitis following treatment occurred. There are three possible reasons for this: either (1) the rapid bactericidal effect of the surgical spirit (Biggs, 1997), or (2) the use of broad spectrum antibiotics, or (3) veterinary administration of the treatments. The use of only one infusion would reduce the risk of introducing infection (Biggs, 1997), however, in this study the treatment of *S. aureus* was also attempted and, therefore, a three day course of treatment was necessary rather than one infusion which may have been sufficient to combat

S. agalactiae.

Bacteriological identification of *S. agalactiae* in this study presented some difficulties, especially after the 'blitz therapy'. The bacteriologists needed to re-check all the *Streptococcus* spp. isolated and the CAMP test was required for accurate identification of the *Streptococcus* spp. The thorough identification process ensured that *S. agalactiae* infection would have been detected had it been present. If the examination process had not been as thorough, there is the possibility that the bacteria may have been missed and the infection could have spread through the herd.

Somatic cells tend to concentrate in the butterfat, as do bacteria (Edmondson, 1998). The method of sampling milk can therefore, produce inaccurate results i.e. artificially high BMSCC and high concentrations of bacteria if the bulk milk is not agitated prior to sampling. Edmondson (1998) found that the BMSCCs could vary by two fold depending on the sampling technique, depending on whether the sample was taken prior to, or after a sufficient period of agitation of the milk in the bulk tank. It was recommended that the milk in the bulk tank is agitated for at least two minutes prior to collection of a sample for BMSCC (Edmondson, 1998) to ensure the milk is adequately mixed. In the current study, bulk milk samples which were taken for bacteriological culturing, and possibly those taken for somatic cell counting, may not have been taken after a standard period of agitation and, therefore, the isolation of bacteria and the BMSCC may have been affected by this.

The IQSCCs were reduced following 'blitz therapy' for almost all the cows. The two cows which showed an increase in IQSCCs to > 400,000 cells/ml may have been approaching drying off, but unfortunately calving dates were not available and so it is impossible to state with any confidence that the increase in IQSCC was an indication of infection. An increase in milk yield would be expected following the treatment of the herd, especially as the IQSCC were seen to decrease, but individual cow milk recording was not performed on this farm. However, the farmer did report that there appeared to be more milk in the bulk tank following the 'blitz therapy' than before the treatment began. Furthermore, the annual milk records for

the farm showed that the quantity of milk sold increased for the quota year following the 'blitz therapy', with the milk sales being very close to the targeted quota for the year (Appendix 8). However, this improvement may not have been solely due to the treatment, as the calving pattern was unknown so that there may have been more cows in early lactation during this period. Other factors such as the quality of feed and the amount of milk taken for feeding to calves may also have contributed to the increased milk sales.

The degree of compliance of the farmer and her ability to follow instructions on improving and making alterations to the milking routine, and implementing the control measures, were major considerations in this study. The 'blitz therapy' was carried out after recommending strict adherence to the 'Five Point Plan' with follow up visits to ensure that the management changes had been made. It was considered that adoption of the recommended management changes was essential in order to safeguard the success of the treatment programme, as poor milking and management practices are known to increase the risk of mastitis (Sandholm and others, 1990). Logue and others (1994) reported that deficiencies in the milking routine resulted in failure to contain spread of infection from cows which were not cured, therefore allowing the continuation of *S. agalactiae* infection, and resulting in the establishment of *S. aureus* infections which were less responsive to antibiotics. These recommendations were used to make improvements to the hygienic practices on the farm and to assess the compliance of the farmer to veterinary advice before investing a considerable amount of money and effort on extensive treatment of the herd. In terms of implementation and follow-up, the more straight-forward total herd 'blitz therapy' regime was chosen instead of the partial herd 'blitz' because it was believed that the farmer was unlikely to be committed to the monitoring necessary for the partial herd 'blitz' regime. Treatments during all studies on this farm were administered by veterinary personnel to ensure that the antibiotics were administered correctly to the right cows.

Economics should always be considered in the management and treatment of disease in farm animals. The whole herd 'blitz therapy' for this herd cost £6,840 which included the costs of investigation, treatment and monitoring. The milk yield for the cows in the herd was assumed

to have increased by 10% after the 'blitz therapy', which would mean that the investment in investigations, treatments and monitoring would have been paid off after 20.5 months due to the increased milk sales alone. Cows were repeatedely screened post-treatment this procedure is not essential and could be omited from the 'blitz therapy' regime when done commercially. The cost of the therapy used was approximately one third of the total cost and therefore, if a less rigorous monitoring protocol was followed, the costs would have been lower still. In order to monitor the response to 'blitz therapy', in most instances it would be sufficient to monitor the BMSCC, along with periodic bacteriological culture of bulk milk samples (McDonald, 1989; Biggs, 1996). If this monitoring procedure had been followed the cost could have been as low as £2,193 (cost of the 'blitz therapy' £1,973 and bulk milk bacteriological examinations £220). The cost of this monitoring programme would be paid off within four months. These calculations do not include the cost of professional time. Professional time priced at £60/hour. Therefore, for the investigations, treatment and monitoring involved in this study herd the cost of professional fees could have amounted to £1,800.

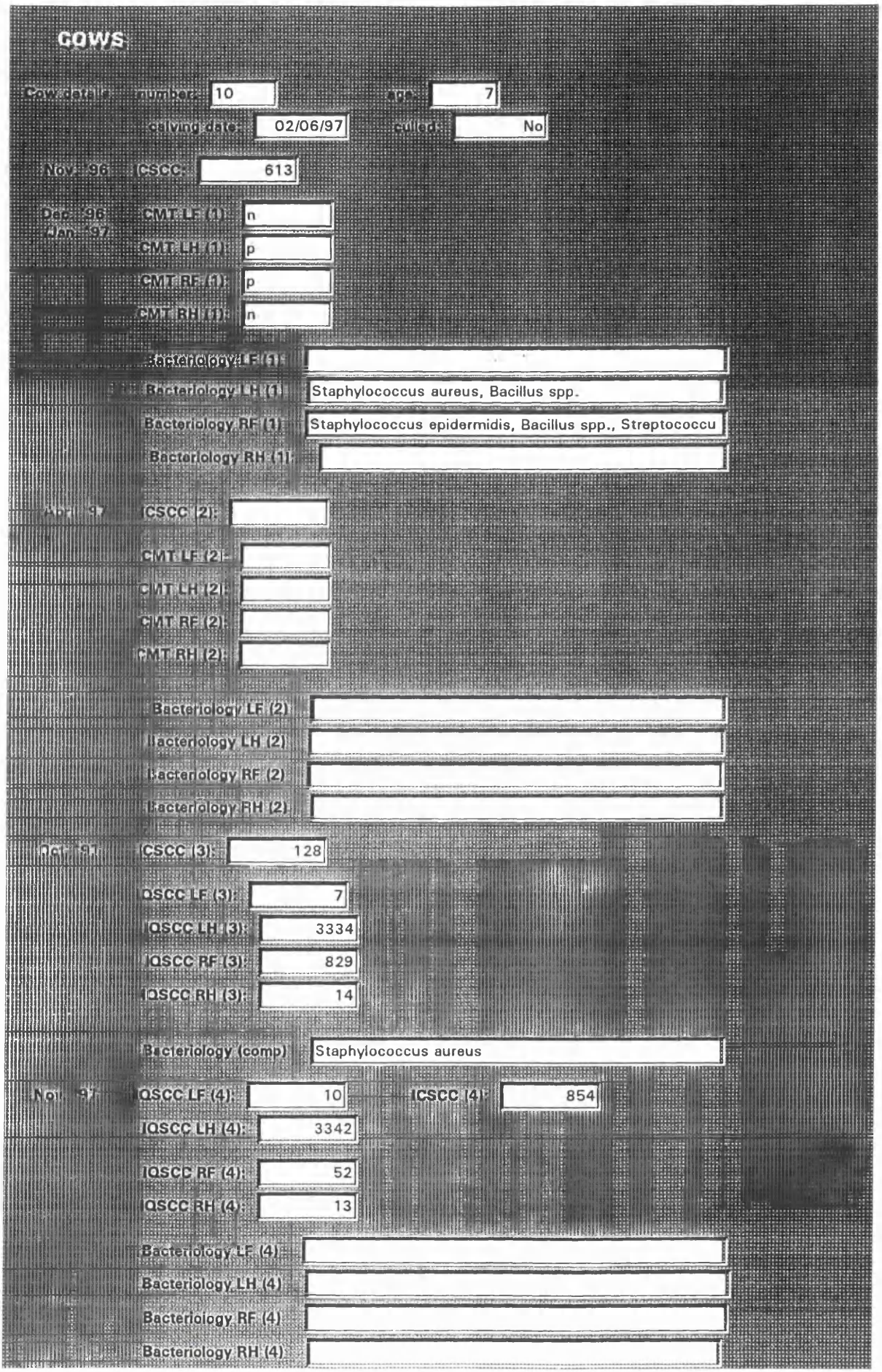
At the time of completion of this study in August 1998, the UK price for milk was decreasing (20 ppl). In addition to the decrease in the milk price, the continued depression in the market for cattle brought about by the BSE crisis has had a considerable impact on the profitability of cattle farming. It is considered likely that consumer pressure will continually demand better quality milk and that this will lead to continued pressure on farmers to reduce SCC and TBC. Taken together, these factors require the farmer to be able to produce milk of high quality in an efficient and cost effective manner. The control of subclinical mastitis is, therefore, an essential area, which as demonstrated in this study, can dramatically increase profitability of farms.

The 'blitz therapy' and control measures would be cost-effective in the study farm after 20.5 months, and the rapid reduction in BMSCC which was maintained for at least one year demonstrates that veterinary involvement was definitely beneficial. The mastitis problem in this dairy farm involved investigation, treatment and careful monitoring. The future for this

farm, however, is still uncertain due to the current economic constraints on the farming industry in general, and due to the continuing high prevalence of *S. aureus* infection in the herd.

Appendix 1

Sample form giving details recorded for each cow


COWS
Cow details
number: 10
age: 7
calving date: 02/06/97
culled: No
Nov. '96
CSCC:
613
Dec. '96
Jan. '97
CMT LF (1):
n
CMT LH (1):
p
CMT RF (1):
p
CMT RH (1):
n
Bacteriology LF (1):
Bacteriology LH (1)
Staphylococcus aureus, Bacillus spp.
Bacteriology RF (1)
Staphylococcus epidermidis, Bacillus spp., Streptococcu
Bacteriology RH (1):
Apr. '97
CSCC (2):
CMT LF (2):
CMT LH (2):
CMT RF (2):
CMT RH (2):
Bacteriology LF (2)
Bacteriology LH (2)
Bacteriology RF (2)
Bacteriology RH (2)
Oct. '97
CSCC (3):
128
QSCC LF (3):
7
QSCC LH (3):
3334
QSCC RF (3):
829
QSCC RH (3):
14
Bacteriology (comp)
Staphylococcus aureus
Nov. '97
QSCC LF (4):
10
CSCC (4):
854
QSCC LH (4):
3342
QSCC RF (4):
52
QSCC RH (4):
13
Bacteriology LF (4)
Bacteriology LH (4)
Bacteriology RF (4)
Bacteriology RH (4)

Appendix 2

Monthly bulk milk somatic cell counts (BMSCC) for quota years 1995-1996 and 1996-1997

Quota Years

1995 - 1996			1996 - 1997		
Year	**Month**	**BMSCC $\times 10^3$ cells/ml**	**Year**	**Month**	**BMSCC $\times 10^3$ cells/ml**
1995	April	478	1996	April	537
	May	358		May	628
	June	385		June	572
	July	352		July	448
	Aug	311		Aug	619
	Sept	310		Sept	562
	Oct	290		Oct	704
	Nov	446		Nov	613
	Dec	495		Dec	735*
1996	Jan	387	1997	Jan	677
	Feb	545		Feb	470**
	March	612		March	502#

* Start of investigations.
** Implementation of improved milking routine.
\# 'Blitz therapy' performed.

Appendix 3

Individual cow somatic cell counts (ICSCC) for those cow lactating during November 1996

Cow ID	ICSCC × 10^3/ml
C5	14
A7	16
D8	17
204	19
D9	31
C6	32
A2	45
140V	57
D16	65
B1	79
58	93
D15	101
D2	149
C15	212
A1	260
C12	282
D5	409
16	480
10	613
C1	703
A6	783
D10	871
A8	1030
A4	1543
35	1812
71	1983
B5	2190
3	2632
D3	3115
C16	3155
5	3292
3P	3866
B8	3941
6	4065
B4	4168
261	4395
28	9714
29	10738
69	18077

Appendix 4

Sample sheets allowing calculation of calving dates

Calving table

Service	Due to calve	Service	Due to calve
Jan 1	Oct 8	Feb 21	Nov 28
2	9	22	29
3	10	23	30
4	11	24	Dec 1
5	12	25	2
6	13	26	3
7	14	27	4
8	15	28	5
9	16	Mar 1	6
10	17	2	7
11	18	3	8
12	19	4	9
13	20	5	10
14	21	6	11
15	22	7	12
16	23	8	13
17	24	9	14
18	25	10	15
19	26	11	16
20	27	12	17
21	28	13	18
22	29	14	19
23	30	15	20
24	31	16	21
25	Nov 1	17	22
26	2	18	23
27	3	19	24
28	4	20	25
29	5	21	26
30	6	22	27
31	7	23	28
Feb 1	8	24	29
2	9	25	30
3	10	26	31
4	11	27	Jan 1
5	12	28	2
6	13	29	3
7	14	30	4
8	15	31	5
9	16	Apr 1	6
10	17	2	7
11	18	3	8
12	19	4	9
13	20	5	10
14	21	6	11
15	22	7	12
16	23	8	13
17	24	9	14
18	25	10	15
19	26	11	16
20	27	12	17

Service	Due to calve	Service	Due to calve
Apr 13	Jan 18	June 8	Mar 15
14	19	9	16
15	20	10	17
16	21	11	18
17	22	12	19
18	23	13	20
19	24	14	21
20	25	15	22
21	26	16	23
22	27	17	24
23	28	18	25
24	29	19	26
25	30	20	27
26	31	21	28
27	Feb 1	22	29
28	2	23	30
29	3	24	31
30	4	25	Apr 1
May 1	5	26	2
2	6	27	3
3	7	28	4
4	8	29	5
5	9	30	6
6	10	31	7
7	11	July 1	8
8	12	2	9
9	13	3	10
10	14	4	11
11	15	5	12
12	16	6	13
13	17	7	14
14	18	8	15
15	19	9	16
16	20	10	17
17	21	11	18
18	22	12	19
19	23	13	20
20	24	14	21
21	25	15	22
22	26	16	23
23	27	17	24
24	28	18	25
25	Mar 1	19	26
26	2	20	27
27	3	21	28
28	4	22	29
29	5	23	30
30	6	24	May 1
31	7	25	2
June 1	8	26	3
2	9	27	4
3	10	28	5
4	11	29	6
5	12	30	7
6	13	31	8
7	14	Aug 1	9

Service	Due to calve	Sevice	Due to calve
Aug 2	May 10	Sep 22	June 29
3	11	23	30
4	12	24	July 1
5	13	25	2
6	14	26	3
7	15	27	4
8	16	28	5
9	17	29	6
10	18	30	7
11	19	Oct 1	8
12	20	2	9
13	21	3	10
14	22	4	11
15	23	5	12
16	24	6	13
17	25	7	14
18	26	8	15
19	27	9	16
20	28	10	17
21	29	11	18
22	30	12	19
23	31	13	20
24	June 1	14	21
25	2	15	22
26	3	16	23
27	4	17	24
28	5	18	25
29	6	19	26
30	7	20	27
31	8	21	28
Sep 1	9	22	29
2	10	23	30
3	11	24	31
4	12	25	Aug 1
5	13	26	2
6	14	27	3
7	15	28	4
8	16	29	5
9	17	30	6
10	18	31	7
11	19	Nov 1	8
12	20	2	9
13	21	3	10
14	22	4	11
15	23	5	12
16	24	6	13
17	25	7	14
18	26	8	15
19	27	9	16
20	28	10	17
21	29	11	18

Service	Due to calve	Service	Due to calve
Nov 12	Aug 19	Dec 7	Sep 13
13	20	8	14
14	21	9	15
15	22	10	16
16	23	11	17
17	24	12	18
18	25	13	19
19	26	14	20
20	27	15	21
21	28	16	22
22	29	17	23
23	30	18	24
24	31	19	25
25	Sep 1	20	26
26	2	21	27
27	3	22	28
28	4	23	29
29	5	24	30
30	6	25	Oct 1
Dec 1	7	26	2
2	8	27	3
3	9	28	4
4	10	29	5
5	11	30	6
6	12	31	7

Appendix 5

Sample sheets for recording reproductive data, mastitis cases and treatments administered

Cow no.	Age of cow	1st service	2nd service	3rd service	Calving date	Mastitis (Y/N) - quarter	Treatment

Appendix 6

Treatments administered during 'blitz therapy'

Cow ID	Treatment administered	Category
3	-	SOLD
5	Depocillin and Nafpenzal	Early lactation (2 months calved)
6	-	SOLD
10	Depocillin and Nafpenzal	Late lactation (calved 2 months after treatment)
16	Synulox	Dry
28	Depocillin and Nafpenzal	Early lactation (calved 8 months after treatment)
29	Depocillin and Nafpenzal	Unknown
35	Depocillin and Nafpenzal	Unknown
57	Depocillin and Nafpenzal	Early lactation (10 days calved)
58	Depocillin and Nafpenzal	Unknown
69	Synulox	Dry (calved 1 month after treatment)
71	Depocillin and Nafpenzal	Unknown
133	Depocillin and Nafpenzal	Early lactation (calved 2.5 months)
140V	Depocillin and Nafpenzal	Unknown
204	Depocillin and Nafpenzal	Early lactation (calved 8 months after treatment)
261	-	SOLD
3P	Synulox	Dry (calved 2 weeks after treatment)
A1	Depocillin and Nafpenzal	Unknown
A2	Depocillin and Nafpenzal	Mid lactation (calved 4 months after treatment)
A4	Depocillin and Nafpenzal	Unknown
A6	Depocillin and Nafpenzal	Late lactation (calved 2 months after treatment)
A7	Depocillin and Nafpenzal	Unknown
A8	Synulox	Dry (calved 2 weeks after treatment)
A9	Depocillin and Nafpenzal	Early lactation (calved 9 months after treatment)
A10	Depocillin and Nafpenzal	Early lactation (calved 2 months)

B1	Depocillin and Nafpenzal	Unknown
B3	Depocillin and Nafpenzal	Early lactation (calved 2 months)
B4	Depocillin and Nafpenzal	Unknown
B5	Synulox	Dry (calved 4 days after treatment)
C1	Synulox	Dry
C2	Depocillin and Nafpenzal	Unknown
C5	Depocillin and Nafpenzal	Unknown
C6	Depocillin and Nafpenzal	Mid lactation (calved 6 months after treatment)
C7	Synulox	Dry (calved 3 weeks after treatment)
C8	Depocillin and Nafpenzal	Unknown
C10	Depocillin and Nafpenzal	Early lactation (calved 7 months after treatment)
C11	Synulox	Dry (calved 6 weeks after treatment)
C12	Synulox	Dry (calved 4 months after treatment)
C15	Depocillin and Nafpenzal	Unknown
C16	Depocillin and Nafpenzal	Mid lactation (calved 5 months after treatment)
D1	Synulox	Dry (calved 1 month after treatment)
D2	Depocillin and Nafpenzal	Mid lactation (calved 4 months after treatment)
D3	Depocillin and Nafpenzal	Unknown
D4	Depocillin and Nafpenzal	Early lactation (calved 2 months)
D5	Depocillin and Nafpenzal	Late lactation (calved 7 months)
D8	Depocillin and Nafpenzal	Late lactation (calved 7 months)
D10	Synulox	Dry (calved 1 month after treatment)
D12	Depocillin and Nafpenzal	Early lactation (calved 2 months)
D14	Depocillin and Nafpenzal	Early lactation (calved 2 months)
D15	Depocillin and Nafpenzal	Early lactation (calved 2 weeks)
D16	Synulox (1 day) then Depocillin and Nafpenzal	Calved on the 3rd day of treatment

Cow ID	Treatment administered	Category
E3	Depocillin and Nafpenzal	Unknown
E4	Depocillin and Nafpenzal	Early lactation (calved 1 week)
E5	Depocillin and Nafpenzal	Unknown
E6	Depocillin and Nafpenzal	Early lactation (calved 1 week)
E7	Depocillin and Nafpenzal	Unknown
E8	Depocillin and Nafpenzal	Unknown
E9	Depocillin and Nafpenzal	Unknown

Unknown - denotes that records were not available to enable the stage of gestation to be calculated, but that the cow was lactating.

Appendix 7

Bacteriology results for all the cows in the herd, highlighting those cows and quarters from which *Staphylococcus aureus* was isolated

Cow ID	Isolation of *Staphylococcus aureus*																	*S. aureus* detected	Selected cows
	Dec-96/Jan-97				Apr-97				Oct-97	Nov-97				Jan-98					
	lf	lh	rf	rh	lf	lh	rf	rh	comp	lf	lh	rf	rh	lf	lh	rf	rh		
3	-	-	-	-	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
5	-	n	n	-	-	n	-	n	-	n	n	n	n	n	n	n	n	No	
6	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
10	-	+	-	+	n	n	n	n	+	n	n	n	n	-	+	-	-	Yes	Yes
16	-	+	n	+	n	n	n	n	+	n	n	n	n	+	+	-	-	Yes	
19	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
28	n	-	n	-	n	n	n	n	n	-	-	+	-	-	-	-	-	Yes	
29	+	-	+	+	-	+	n	-	n	n	n	n	n	n	n	n	n	Yes	
35	+	n	n	n	n	n	n	n	+	n	n	n	n	n	n	n	n	Yes	
57	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
58	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
60	-	n	-	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
69	+	n	-	n	n	n	n	n	-	n	n	n	n	n	n	n	n	Yes	
71	n	-	-	n	+	n	n	n	+	n	n	n	n	n	n	n	n	Yes	
133	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
140V	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	n	No	
169	n	n	n	n	n	n	n	n	n	n	n	n	n	-	-	-	-	No	
204	-	+	-	-	n	n	n	n	n	-	-	-	-	n	n	n	n	Yes	
261	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
3P	n	n	n	-	-	+	n	-	-	n	n	n	n	-	+	-	-	Yes	
A1	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
A2	n	n	n	n	-	-	-	-	-	n	n	n	n	n	n	n	n	No	

Cow ID	Isolation of *Staphylococcus aureus*																	*S. aureus* detected	Selected cows
	Dec-96/Jan-97				Apr-97				Oct-97	Nov-97				Jan-98					
	lf	lh	rf	rh	lf	lh	rf	rh	comp	lf	lh	rf	rh	lf	lh	rf	rh		
A4	+	+	-	+	-	-	-	-	n	n	n	n	n	n	n	n	n	Yes	
A6	n	n	n	-	n	n	n	n	+	n	n	n	n	+	n	n	+	Yes	Yes
A7	n	n	n	n	n	-	n	n	n	n	n	n	n	n	n	n	n	No	
A8	n	n	n	-	n	+	n	-	+	n	n	n	n	n	n	n	n	Yes	
A9	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	n	No	
A10	n	n	n	n	n	n	n	n	-	n	n	n	n	-	+	-	-	Yes	
B1	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
B3	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
B4	n	-	-	n	-	-	-	-	n	n	n	n	n	n	n	n	n	No	
B5	n	-	+	n	+	+	-	-	n	n	n	n	n	n	n	n	n	Yes	
B8	n	n	n	n	n	n	n	n	n	+	-	-	-	n	n	n	n	Yes	
C1	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
C2	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
C5	-	-	-	-	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
C6	n	n	n	n	-	-	-	n	-	n	n	n	n	n	n	n	n	No	
C7	n	n	n	n	-	n	n	n	-	n	n	n	n	n	n	n	n	No	
C8	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
C10	n	n	n	n	n	n	n	n	n	-	+	-	-	-	+	-	-	Yes	
C11	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
C12	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
C15	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
C16	n	n	-	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	

Cow ID	Isolation of *Staphylococcus aureus*																	*S. aureus* detected	Selected cows
	Dec-96/ Jan-97				Apr-97				Oct-97	Nov-97				Jan-98					
	lf	lh	rf	rh	lf	lh	rf	rh	comp	lf	lh	rf	rh	lf	lh	rf	rh		
D1	n	n	n	n	n	n	n	n	+	n	n	n	n	-	-	-	-	Yes	
D2	n	n	n	n	-	n	n	n	-	n	n	n	n	n	n	n	n	No	
D3	n	n	n	n	-	-	-	-	n	n	n	n	n	n	n	n	n	No	
D4	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
D5	n	-	n	n	n	-	n	-	n	-	+	-	+	-	+	-	-	Yes	Yes
D8	-	-	-	+	n	n	n	n	n	-	-	-	-	n	n	n	n	Yes	
D9	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
D10	n	n	n	n	n	n	n	n	-	n	n	n	n	+	+	-	-	Yes	
D12	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
D14	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
D15	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
D16	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
E1	n	n	n	n	n	n	n	n	n	-	-	-	-	-	-	-	-	No	
E3	n	n	n	n	n	-	n	-	n	n	n	n	n	n	n	n		No	
E4	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
E5	n	n	n	n	n	n	n	n	-	n	n	n	n	-	n	n	n	No	
E6	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
E7	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
E8	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
E9	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	No	
E10	n	n	n	n	n	n	n	n	-	n	n	n	n	n	n	n	n	No	
F1	n	n	n	n	n	n	n	n	n	-	-	-	-	-	-	-	-	No	

Cow ID	Isolation of *Staphylococcus aureus*																	*S. aureus* detected	Selected cows
	Dec-96/ Jan-97				Apr-97				Oct-97	Nov-97				Jan-98					
	lf	lh	rf	rh	lf	lh	rf	rh	comp	lf	lh	rf	rh	lf	lh	rf	rh		
F2	n	n		n	n	n	n	n	n	-	-	-	-	-	-	-	-	No	
F3	n	n	n	n	n	n	n	n	n	+	+	-	+	-	-	n	-	Yes	Yes
F4	n	n	n	n	n	n	n	n	n	-	-	-	-	-	-	-	-	No	
F5	n	n	n	n	n	n	n	n	n	n	n	n	n	-	-	-	-	No	
F6	n	n	n	n	n	n	n	n	n	-	+	-	+	-	+	-	-	Yes	Yes
F8	n	n	n	n	n	n	n	n	n	n	n	n	n	-	-	-	-	No	
F9	n	n	n	n	n	n	n	n	n	n	n	n	n	-	-	+	-	Yes	
F10	n	n	n	n	n	n	n	n	n	n	n	n	n	-	+	-	+	Yes	Yes

+ indicates that *Staphylococcus aureus* was isolated
- indicates that *Staphylococcus aureus* was not isolated
n indicates that a sample was not collected for examination
comp. - composite milk sample

lf - left front quarter
lh - left hind quarter
rf - right front quarter
rh - right hind quarter

Appendix 8

Details of the milk sales for the last three quota years

tre)	Bfat	Prot	Lact	TBC	SCC	Value	TBC Amt	SCC Amt
.463	3.76	3.07	4.55	10	574	2,813.31	24.93	-24.93
.315	3.38	3.22	4.66	7	502	3,863.57	34.63	-34.63
.869	3.72	3.44	4.67	7	401	3,870.79	33.74	-33.74
.441	3.68	3.52	4.62	7	362	3,392.43	28.88	
.620	3.85	3.35	4.65	7	350	2,741.35	23.24	
.286	3.94	3.57	4.64	7	323	3,268.22	26.57	
.051	4.39	3.45	4.65	6	298	3,089.95	24.10	
.644	4.27	3.11	4.72	6	326	3,200.38	25.29	
.359	4.21	3.03	4.76	7	368	3,813.07	30.72	
.774	4.11	2.95	4.70	7	402	3,283.68	27.55	-55.10
.396	4.13	3.05	4.76	9	437	3,499.69	28.79	-57.58
.371	4.03	3.10	4.76	10	487	3,487.94	28.74	-57.48

to Screen Period				Year to Date (1995)			
85,994				Supply:	168.589		
3.70	TBC Avg:	8	Bfat Avg:		3.94	TBC Avg:	8
3.36	SCC Avg:	419	Prot Avg:		3.24	SCC Avg:	403

t Haulage Average Next Prev Next-Yr Prev-Yr Detail Exit

Scottish Milk Ltd. Welcome to DairyTrend 14/09/98

Yearly Enquiry

Farm Code: Name. 133

Mth	Vol (Litre)	Bfat	Prot	Lact	TBC	SCC	Value	TBC Amt	SCC Amt
Apr	13,440	4.05	3.02	4.69	10	558	3,008.39	26.88	-134.40
May	17,000	3.82	3.19	4.69	10	590	3,488.84	34.00	-170.00
Jun	16,059	3.65	3.40	4.79	12	576	3,176.45		-160.59
Jul	15,281	3.72	3.41	4.68	12	536	4,003.19	30.56	-152.81
Aug	10,783	3.75	3.36	4.55	10	530	2,713.13	21.57	-107.83
Sep	10,832	3.96	3.64	4.56	13	522	2,909.85	21.66	-108.32
Oct	9,683	4.28	3.49	4.54	16	609	2,549.82		-96.83
Nov	10,247	4.13	3.27	4.60	16	613	2,514.10		-102.47
Dec	10,295	3.95	3.12	4.61	15	672	2,260.14		-102.95
Jan	11,091	3.94	3.07	4.66	15	658	2,186.93		-332.73
Feb	10,494	3.94	3.02	4.72	10	595	2,068.22	20.99	-314.82
Mar	9,048	3.98	2.90	4.70	9	502	1,701.50	18.10	-271.44

Year to Screen Period				Year to Date (1996)			
Supply:	83,395			Supply:	144,253		
Bfat Avg:	3.82	TBC Avg:	11	Bfat Avg:	3.91	TBC Avg:	12
Prot Avg:	3.32	SCC Avg:	552	Prot Avg:	3.25	SCC Avg:	580

Units Stat Haulage Average Next Prev Next-Yr Prev-Yr Detail Exit

Yearly Enquiry

Farm Code: Name: 134

Mth	Vol (Litre)	Bfat	Prot	Lact	TBC	SCC	Value	TBC Amt	SCC Amt
Apr	13,209	3.90	2.90	4.76	7	365	2,532.19	39.63	
May	19,141	3.73	3.20	4.71	6	281	3,338.14	57.42	
Jun	18,764	3.81	3.42	4.90	4	245	3,484.40	56.29	18.76
Jul	15,402	3.84	3.47	4.88	4	242	3,543.87	46.21	15.40
Aug	14,630	3.84	3.42	4.87	7	232	3,530.27	43.89	14.63
Sep	12,525	4.00	3.60	4.77	6	209	3,153.89	37.58	12.53
Oct	10,015	4.50	3.64	4.77	5	162	2,431.61	30.05	10.02
Nov	10,356	4.38	3.34	4.72	7	155	2,328.06	31.07	10.36
Dec	12,564	4.08	3.05	4.85	6	156	2,432.41	37.69	12.56
Jan	11,376	3.72	2.88	4.65	5	184	2,051.06	34.13	11.38
Feb	11,370	3.71	2.85	4.80	8	250	2,036.30	34.11	11.37
Mar	12,593	3.76	2.86	4.82	5	323	2,196.29	37.78	

Year to Screen Period				Year to Date (1997)			
Supply:	93,671			Supply:	161.945		
Bfat Avg:	3.84	TBC Avg:	6	Bfat Avg:	3.91	TBC Avg:	6
Prot Avg:	3.33	SCC Avg:	262	Prot Avg:	3.23	SCC Avg:	234

Units Stat Haulage Average Next Prev Next-Yr Prev-Yr Detail Exit

Scottish Milk Ltd. Welcome to DairyTrend 14/09/98

Yearly Enquiry

135

Farm Code: Name:

Mth	Vol (Litre)	Bfat	Prot	Lact	TBC	SCC	Value	TBC Amt	SCC Amt
Apr	12,152	3.70	2.84	4.81	6	352	1,851.98	36.46	-60.76
May	16,053	3.64	2.89	4.77	7	326	2,133.38	48.16	
Jun	17,115	3.70	3.35	4.93	7	318	2,555.26	51.35	
Jul	16,220	3.94	3.43	4.90	18	338	3,381.96		
Aug	13,685	4.33	3.40	4.81	11	377	2,872.54		-68.43
Sep	4,032	3.86	3.41	4.80	6	371	0.00	12.10	-20.16
Oct							0.00		
Nov							0.00		
Dec							0.00		
Jan							0.00		
Feb							0.00		
Mar							0.00		

Year to Screen Period				Year to Date (1998)			
Supply:	79,257			Supply:	79,257		
Bfat Avg:	3.85	TBC Avg:	9	Bfat Avg:	3.85	TBC Avg:	9
Prot Avg:	3.21	SCC Avg:	347	Prot Avg:	3.21	SCC Avg:	347

Units Stat Haulage Average Next Prev Next-Yr Prev-Yr Detail Exit

Select display units

Farm Header Enquiry

Farm Code: Year: 1995 Base Bfat: 3.840 Adj. Bfat: 3.850000

Mth	Milk Supply Litres	Butter-fat	Profile Details	Quota Litres	Butterfat Adjustment	Adjusted Supply
Apr	12,463	3.760	8.2191	15,067	202-	12,261
May	17,315	3.380	8.4932	15,569	1,465-	15,850
Jun	16,869	3.720	8.2191	15,067	395-	16,475
Jul	14,441	3.680	8.4932	15,569	442-	13,999
Aug	11,620	3.850	8.4932	15,569	0	11,620
Sep	13,286	3.940	8.2191	15,067	215	13,501
Oct	12,051	4.390	8.4932	15,569	1,171	13,223
Nov	12,644	4.270	8.2191	15,067	956	13,600
Dec	15,359	4.210	8.4932	15,569	995	16,354
Jan	13,774	4.110	8.4932	15,569	645	14,418
Feb *	13,917	4.130	7.6712	14,062	701	14,619
Mar	14,371	4.030	8.4932	15,569	466	14,837
YTD.	168,112	3.940	100.0000	183,311	2,723	170,835
YTD Var			(-8.29%)	15,199-	(-6.85%)	12,553-
Annual	168,112	3.940		183,311		
Ann Var			(-8.29%)	15,199-	(-6.85%)	12,476-

arm: Leap Adj.: 479

Next Prev Next-Yr Prev-yr Quality Exit

Display previous year

Farm Header Enquiry

Farm Code: Year: 1996 Base Bfat: 3.840 Adj. Bfat: 3.850000

Mth	Milk Supply Litres	Butter-fat	Profile Details	Quota Litres	Butterfat Adjustment	Adjusted Supply
Apr	13,440	4.050	7.3926	13,551	484	13,924
May	17,000	3.820	10.2706	18,827	92-	16,908
Jun	16,059	3.650	10.0061	18,342	578-	15,481
Jul	15,281	3.720	8.5659	15,702	358-	14,924
Aug	10,783	3.750	6.8926	12,635	194-	10,589
Sep	10,832	3.960	7.8806	14,446	214	11,047
Oct	9,683	4.280	7.1482	13,103	749	10,432
Nov	10,247	4.130	7.4996	13,748	516	10,764
Dec	10,295	3.950	9.1103	16,700	185	10,480
Jan	11,091	3.940	8.1699	14,976	180	11,270
Feb	10,494	3.940	8.5391	15,653	170	10,664
Mar	9,048	3.980	8.5245	15,626	212	9,260
YTD.	144,252	3.910	100.0000	183,311	1,558	145,810
YTD Var			(-21.31%)	39,059-	(-20.49%)	37,570-
Annual	144,252	3.910		183,311		
Ann Var			(-21.31%)	39,059-	(-20.49%)	37,501-

Farm:

Next Prev Next-Yr Prev-yr Quality Exit

Display previous year

Farm Header Enquiry

Farm Code: Year: 1997 Base Bfat: 3.840 Adj. Bfat: 3.856242

Mth	Milk Supply Litres	Butter-fat	Profile Details	Quota Litres	Butterfat Adjustment	Adjusted Supply
Apr	13,209	3.900	9.3169	15,216	95	13,304
May	19,141	3.730	11.7846	19,246	448-	18,693
Jun	18,764	3.810	11.1325	18,181	169-	18,595
Jul	15,402	3.840	10.5933	17,300	55-	15,346
Aug	14,630	3.840	7.4748	12,207	53-	14,578
Sep	12,525	4.000	7.5092	12,263	316	12,841
Oct	10,015	4.500	6.7125	10,962	1,154	11,169
Nov	10,356	4.380	7.1038	11,601	969	11,325
Dec	12,564	4.080	7.1367	11,655	498	13,062
Jan	11,376	3.720	7.6882	12,556	287-	11,089
Feb	11,370	3.710	7.2746	11,880	307-	11,063
Mar	12,593	3.760	6.2729	10,244	227-	12,367
YTD	161,944	3.910	100.0000	163,311	1,457	163,402
YTD Var			(-0.84%)	1,367-	(0.07%)	119
Annual	161,944	3.910		163,311		
Ann Var			(-0.84%)	1,367-	(0.07%)	91

Farm:

Next Prev Next-Yr Prev-yr Quality Exit

Display previous year

Farm Header Enquiry

Farm Code: Year: 1998 Base Bfat: 3.840 Adj. Bfat: 3.856242

Mth	Milk Supply Litres	Butter-fat	Profile Details	Quota Litres	Butterfat Adjustment	Adjusted Supply
Apr	12,152	3.700	8.1565	14,952	350-	11,802
May	16,053	3.640	11.8193	21,666	636-	15,417
Jun	17,115	3.700	11.5864	21,239	493-	16,622
Jul	16,220	3.940	9.5105	17,434	234	16,454
Aug	13,685	4.330	9.0341	16,561	1,158	14,843
Sep	4,032	3.860	7.7343	14,178	0	4,032
Oct	0	0.000	6.1841	11,336	0	0
Nov	0	0.000	6.3948	11,722	0	0
Dec	0	0.000	7.7583	14,222	0	0
Jan	0	0.000	7.0245	12,877	0	0
Feb	0	0.000	7.0207	12,870	0	0
Mar	0	0.000	7.7765	14,255	0	0
YTD.	79,257	3.850	100.0000	96,105	143-	79,114
YTD Var			(-17.53%)	16,848-	(-17.62%)	16,935-
Annual	0	3.850		183,311		
Ann Var			(-56.76%)	104,054-	(-56.81%)	104,197-

Farm:

Next Prev Next-Yr Prev-yr Quality Exit

Display next record

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