



Bovine Mastitis: An Evolving Disease

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SUMMARY

Mastitis remains a major challenge to the worldwide dairy industry despite the widespread implementation of mastitis control strategies. The last forty years have seen a dramatic decrease in clinical mastitis incidence but this has been accompanied by a change in the relative and absolute importance of different pathogens. *Escherichia coli* and *Streptococcus uberis* are now the two most common causes of bovine mastitis and are an increasing problem in low somatic cell count herds. This paper reviews the changes in incidence and pattern of mastitis in the UK over the last four decades and discusses some of the possible explanations for these changes. It focuses in particular on apparent changes in the behaviour of *E. coli* and its ability to cause persistent intramammary infection; which may be as a result of bacterial adaptation or the unmasking of previously unrecognized patterns of pathogenesis. The prospects for novel approaches to mastitis control are discussed, as are the current and future challenges facing the industry.

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INTRODUCTION

Bovine mastitis, defined as 'inflammation of the mammary gland', can have an infectious or non-infectious aetiology. Organisms as diverse as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease; Watts (1988) identified 137 different organisms as a cause of mastitis. Fortunately the vast majority of mastitis in the UK is of bacterial origin and just five species of bacteria (*Escherichia coli*, *Streptococcus uberis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae*) account for almost 80% of all diagnoses (Anon., 2001).

Classically, mastitis pathogens have been classified as either 'contagious' or 'environmental' (Blowey & Edmondson, 1995). In essence, the contagious pathogens can be considered as organisms adapted to survive within the host, in particular within the mammary gland. They are capable of establishing sub-clinical infections, which are typically manifest as an elevation in the somatic cell count (leukocytes

[predominantly neutrophils] and epithelial cells) of milk from the affected quarter; they are typically spread from cow to cow at or around the time of milking (Radostits *et al.*, 1994). In contrast, the environmental pathogens are best described as opportunistic invaders of the mammary gland, not adapted to survival within the host; typically they 'invade', multiply, engender a host immune response and are rapidly eliminated. The major contagious pathogens comprise *S. aureus*, *Str. dysgalactiae* and *Str. agalactiae*; the major environmental pathogens comprise the *Enterobacteriaceae* (particularly *E. coli*) and *Str. uberis*. However, there is now an increasing body of evidence, discussed later in this review, to suggest that this classification may not be as clear cut as previously thought.

The importance of mastitis

Mastitis continues to be the most economically important disease of dairy cattle, accounting for 38% of the total direct costs of the common production diseases (Kossaibati & Esslemont, 1997). It is notoriously difficult to estimate the losses associated with clinical mastitis, which arise from the costs of treatment, culling, death and decreased milk production.

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The average cost of a case of clinical mastitis has recently been estimated to be £175 (Kossaibati, 2000). Assuming an average incidence of 40 cases/100 cows/year and a national herd size of 2.4 million dairy cows, clinical mastitis costs the UK dairy industry in excess of £168 million annually. In a recent study, the annual mortality rate as a result of mastitis was 0.6% of lactating cows (Bradley & Green, 2001a). Mastitis has also been identified as the most common cause of death in adult dairy cows (Esslemont & Kossaibati, 1997).

It is even more difficult to quantify the losses associated with sub-clinical mastitis, which arise as a result of treatment, decreased milk yield and constituent quality, and an increase in the risk of culling. Philpott (1984) demonstrated an inverse linear relationship between somatic cell count (SCC) above 200 000 cells/ml and yield (2.5% decrease in yield for each 100 000 cells/ml increase in SCC). Other workers have demonstrated apparent benefits of reducing SCCs below 90 000 cells/ml (Deluyker *et al.*, 1993). In addition to the costs outlined above, both clinical and sub-clinical mastitis have been shown to adversely affect subsequent fertility (Schrick *et al.*, 2001).

As well as the financial implications of mastitis, the importance of mastitis in public health should not be overlooked. The extensive use of antibiotics in the treatment and control of mastitis has possible implications for human health through an increased risk of antibiotic resistant strains of bacteria emerging that may then enter the food chain (White & McDermott, 2001). The potential spread of zoonotic organisms via milk, though rare in the era of pasteurisation, remains a risk especially in the niche markets of unpasteurised dairy products, and during pasteurisation failures.

The welfare implications of mastitis were highlighted in the UK Farm Animal Welfare Council Report on the Welfare of Dairy Cattle (FAWC, 1997). The welfare implications of per-acute toxic mastitis are obvious; more recent research, however, has demonstrated significant secondary hyperalgesia in cows following mild clinical episodes of mastitis (Fitzpatrick *et al.*, 1998).

THE CHANGING EPIDEMIOLOGY OF BOVINE MASTITIS

Historical perspective

In the 1940s the average herd size in the UK was about 15 cows, which would have had an estimated

23 cases of mastitis annually due mainly to *Str. agalactiae* and *S. aureus*. The average somatic cell count was probably around 750 000 cells/ml (Booth, 1997). However, there was great optimism that penicillin was about to eradicate mastitis, but it was not until the 1960s that real progress was made in the control of the disease. It was at this time that the Five-Point Plan was devised from research at the National Institute for Research in Dairying (NIRD) in Reading (Neave *et al.*, 1966; Smith *et al.*, 1967; Neave *et al.*, 1969; Kingwill *et al.*, 1970). The plan called for a five-pronged approach to the management of mastitis, namely rapid identification and treatment of clinical cases, routine whole herd antibiotic dry cow therapy, post milking teat disinfection, culling of chronically affected cows and the routine maintenance of the milking machine. It was the uptake of this plan that resulted in rapid progress in control of both clinical and sub-clinical mastitis in the UK. In addition to the initial benefit of implementation of the Five-Point Plan, a number of factors have added impetus to UK mastitis control programmes. The most notable of these were the implementation of EC Milk Hygiene Directive (92/46) that imposed an upper limit of 400 000 cells/ml in bulk milk for human consumption and the economic incentives offered to farmers, by milk purchasers, to producer milk of higher quality with a lower SCC.

The impact of the implementation of mastitis control strategies, and in particular the Five-Point Plan, has been very successful in controlling the contagious pathogens and has led to a massive reduction in the incidence of clinical and sub-clinical mastitis and bulk milk somatic cell counts (BMSCC). The historical change in clinical mastitis incidence and its causes is illustrated in Table I.

Table I
Incidence and aetiology of clinical mastitis in UK dairy herds (Quarter cases/100 cows/year)

<i>Pathogen</i>	1967 ^a	1982 ^b	1998 ^c
<i>Staphylococcus aureus</i>	67	7	2.2
<i>Streptococcus agalactiae</i>	6	1	–
<i>Streptococcus dysgalactiae</i>	16	4	2.0
<i>Streptococcus uberis</i>	7	9	5.3
<i>Escherichia coli</i>	7	10	14.4
Other	50	9	17.7
Total	153	40	41.6

^aWilson & Kingwill, 1975, ^bWilesmith *et al.*, 1986, ^cBradley & Green, 2001.

Table II
Annual bulk milk somatic cell counts—proportion
(%) of herds falling within different ranges

Cell Count ($\times 10^3$)	1979 ^a	1993 ^a	2001 ^b
<200	2	26	71
200–399	35	47	26
>400	63	27	3

^aBooth, 1997, ^bM. Blanshard, personal communication, 2001.

Between 1967 and 1982 there was a dramatic decrease in both the overall incidence of mastitis, which fell from over 150 (Wilson & Kingwill, 1975) to 40 (Wilesmith *et al.*, 1986) cases per 100 cows per year and in the incidence of the contagious mastitis pathogens. Over the same time period the average BMSCC in England and Wales fell from over 600 000 cells/ml to just over 400 000 cells/ml (Booth, 1997). This fall is reflected in the distribution of herds between different cell count bands as illustrated in Table II (Booth, 1997; M. Blanshard, Personal communication, 2001).

The introduction of antibiotic dry cow therapy, which had been demonstrated to reduce summer mastitis (Pearson, 1950, 1951) would have resulted in a dramatic decrease in the incidence of summer mastitis. However, in contrast to the other major mastitis pathogens there has been little change in the incidence of summer mastitis over recent years (Booth, 1997; Berry & Booth, 1999).

Current situation

There is a dearth of recent information on the incidence and aetiology of mastitis in the UK. The annual Veterinary Investigation Diagnosis Analysis Database (VIDA) statistics compiled by the Veterinary Laboratories Agency give an insight into the current profile of mastitis pathogens, although unfortunately, this data is unlikely to give a true reflection of the field situation, as samples submitted to the laboratory are likely to be biased towards problem herds and cows rather than reflecting the population as a whole. One recent study suggested a clinical mastitis incidence of between 30 and 40 cases/100 cows/year (Berry, 1998). Retrospective analysis of the data from 144 farms for the years 1994–1996 gave a figure of 43.4 cases/100 cows/year (Kossabati *et al.*, 1998). The only recent published prospective study that attempted to measure incidence and aetiology of mastitis in the UK studied six herds and found an incidence of 41.6 cases/100

cows/year (Bradley & Green, 2001a), which correlates well with recent retrospective analyses.

Data from the aforementioned prospective study (Bradley & Green, 2001a) on mastitis aetiology is compared with historical figures in Table I. *E. coli* was the commonest cause of mastitis, being implicated as the causal organism in 34.7% of clinical cases. *Enterobacteriaceae* were responsible for 40.9% of all mastitis. Contagious pathogens (*S. aureus*, *Str. dysgalactiae*, *Str. agalactiae*) accounted for only 10% of clinical cases. Recent VIDA statistics have been subdivided into those from clinical and sub-clinical mastitis. These statistics confirm *E. coli* (27%) as the commonest cause of clinical mastitis closely followed by *Str. uberis* (23%); contagious pathogens were only implicated in 18% of cases (Anon., 2001). These figures confirm the importance of the environmental pathogens, especially when one considers that samples submitted to laboratories tend to be biased towards problem cows and herds.

Current data on sub-clinical mastitis is even less readily available. Data from the National Milk Records Service (NMR) suggested that the average BMSCC in the UK was approximately 170 000 cells/ml in January 2001 (M. Blanshard, Personal communication, 2001). The distribution of herds towards low somatic cell count bands has progressed since the early 1990s (see Table II) to the point where 21% and 71% of recorded herds had BMSCCs below 100 000 and 200 000 cells/ml respectively in January 2001 (M. Blanshard, personal communication, 2001). During 2001 there was an upward trend in BMSCC, as reported by NMR; it was hypothesised that this rise was due to a reduction in culling of infected cows as a result of the 2001 Foot-and-Mouth epidemic. The most recent VIDA statistics, compiled for the year 2000, confirm *S. aureus* as the most common cause of sub-clinical mastitis in the UK (Anon., 2001). However, *Str. uberis* and *E. coli* accounted for 24% and 12% of diagnoses respectively and between them are as prevalent as the major contagious pathogens combined. These data need to be interpreted with caution, as individual cow histories and the security of sample collection are difficult to verify. These findings are, however, supported by a prospective study on six low BMSCC dairy herds in Somerset which identified *E. coli* as the most prevalent pathogen in clinically normal cows sampled at drying off (Bradley & Green, 2000), affecting 2.14% of quarters.

In summary, since the implementation of the Five-Point Plan there has been a dramatic decrease in BMSCCs, clinical mastitis and the importance of

the contagious mastitis pathogens. However, over the same time period there has been an absolute as well as relative increase in the incidence of environmental pathogens. *S. aureus* continues to be a major cause of sub-clinical mastitis though there appears to be some evidence that pathogens previously considered to be purely environmental may also be capable of causing persistent infection. These changes in aetiology and some possible explanations for these changes are discussed later in this paper.

FACTORS INFLUENCING THE CHANGING INCIDENCE AND AETIOLOGY OF MASTITIS

Considering the increasing demands put on the modern dairy cow, one would have expected an increase in the incidence of clinical mastitis in the UK dairy herd. The fact that this has not happened gives credit to the success of the control strategies adopted as part of the Five-Point Plan. An in-depth discussion of all the factors influencing mastitis incidence and aetiology is beyond the scope of this review. The remainder of this paper will focus on the incidence and aetiology of mastitis in low BMSCC herds and on possible reasons for the pattern of disease seen in these herds.

Mastitis in low cell count herds

The traditional concept of environmental mastitis is that the organisms live in the environment and contaminate the teats. Invasion of the udder is considered to occur when the teat orifice is open, e.g. at or soon after milking or after teat damage. Following rapid bacterial multiplication in the milk, an inflammatory response is mounted. The severity of the disease is then thought to be influenced in part by the speed of the immune response, in particular polymorphonuclear cell migration into the udder (Hill, 1981; Shuster *et al.*, 1996; Van Werven, 1999). Control of the disease has centred on reducing environmental challenge around parturition and during lactation and ensuring strict hygiene at and after milking.

The mystery of environmental mastitis has been that while herds have been able to make management changes to reduce the amount of contagious mastitis, husbandry improvements have apparently not resulted in a reduction of environmental infections. It has been found that even some well managed herds, which can maintain low somatic cell counts (SCC), find it difficult to control environmental mastitis (Hogan *et al.*, 1989), and may even experience a higher incidence of disease (Erskine

et al., 1988; Miltenburg *et al.*, 1996). Why is it that herds that are capable of reducing contagious mastitis seem less able to reliably prevent environmental mastitis? Are they unable/unprepared to 'clean up' sufficiently, is it impossible to reduce environmental challenge by the necessary amount in day-to-day farming conditions, or is the situation more complicated than this?

A recent UK questionnaire-based survey investigated the incidence of mastitis in herds with a mean BMSCC below 100 000 cells/ml in the previous 12 months (Peeler *et al.*, 2000). This study reported a mean clinical mastitis incidence of 22.8 cases/100 cows/year, in the 1771 herds responding to the questionnaire, well below the UK average of around 40 cases/100 cows/year (Bradley & Green, 2001a). However, there was a wide variation in the reported incidence on these farms from 0.6 to 147.1 cases/100 cows/year implying that it is possible to have a low incidence of clinical mastitis in low BMSCC herds. This study (Peeler *et al.*, 2000) identified a number of risk factors for increased incidence of clinical mastitis; unsurprisingly the quality of environmental management featured strongly in the results though these and other factors identified need further investigation.

The lack of success in reducing *E. coli* mastitis has led to questions being asked concerning other risk factors for the disease. Various studies have implicated factors that may increase the likelihood of *E. coli* mastitis, and these have been reviewed elsewhere (Jones, 1990); some of these factors are discussed below.

A Comparison of high and low cell count cows and herds

At the cow level, a number of studies have found that mastitis generally increases as SCCs increase (Coffey *et al.*, 1986; Deluyker *et al.*, 1993; Beaudeau *et al.*, 1998; Rupp *et al.*, 2000), although this may not be surprising because the type of pathogen was not specified and higher cell count cows are likely to be persistently infected with contagious pathogens and therefore be prone to recurrent bouts of mastitis. However, Hogan *et al.* (1989) studied mastitis in herds where contagious pathogens had been controlled, as measured by SCC. The criteria used for inclusion in the study was that >80% of the herd had a Dairy Herd Improvement (DHI) linear somatic cell count score <5. This criterion was deemed to be effective at selecting herds that had controlled contagious pathogens. In these herds he found that >80% of the mastitis was due to environmental pathogens.

Other studies have used BMSCCs of less than 150 000 cells/ml and found a higher incidence of mastitis in low BMSCC as compared to high BMSCC herds (Erskine *et al.*, 1988). They also found an increased level of coliform mastitis in the low BMSCC group as illustrated in Table III. These findings were supported by a more recent study in the Netherlands (Miltenburg *et al.*, 1996) where a significant increase in mastitis (with the most common pathogen being *E. coli*) was associated with a reduction in BMSCC (<150 000 cf. >250 000). Barkema *et al.* (1998) also found that *E. coli* infections were more common in low cell count herds, but overall there was no association between mastitis incidence and BMSCC. However, they did find the highest variation in incidence rate and a higher incidence of mastitis with systemic signs in the low cell count herds (<150 000 cells/ml). Other workers have also found an association between low BMSCC and an increase in the incidence of toxic mastitis (Green *et al.*, 1996; Tadich *et al.*, 1998). More recently, a single herd study of cows with SCCs below 200 000 cells/ml identified cows with a lower somatic cell count as being more likely to succumb to clinical mastitis in the subsequent month (Suriyasathaporn *et al.*, 2000).

A retrospective study on two farms (unpublished data) found that cows were at reduced risk of developing clinical coliform mastitis in early lactation if they had had a mean somatic cell count between 20 000 and 60 000 cells/ml in the previous lactation when compared to cows which had had mean counts below 20 000 or above 60 000 cells/ml. These data suggest that there may be a 'U' shaped distribution of risk with both very low and also elevated individual cow SCCs predisposing to clinical mastitis, as a result of an inadequate immune response or inherent susceptibility to infection

respectively, whereas cows with an intermediate somatic cell count may be relatively protected. This phenomenon has been described by a number of authors and has recently been reviewed (Schukken *et al.*, 2001).

Somatic cell counts – experimental evidence

Somatic cells in milk are a pivotal part of mammary gland immunity and are vital for protection against intramammary infection. The predominant cell type present in the milk of uninfected quarters is the macrophage (Lee *et al.*, 1980): Through the secretion of chemokines, macrophages are responsible for recruiting the large numbers of neutrophils which predominate in infected glands (Sordillo *et al.*, 1997) and which are required to fight infection at the time of intramammary challenge. The exact role of somatic cells in the control of clinical mastitis has been an area of hot debate over a number of years as has the relative importance of absolute numbers of cells present in milk, compared to the speed of recruitment of additional cells following challenge. A vital component of any study trying to investigate this phenomenon has to be the identification of the aetiological agent involved. There is a well recognised association between high SCCs and contagious pathogens and it is only by removing this from the equation that one can hope to identify any potential increase in the risk of environmental mastitis as somatic cell count falls. Some key studies that provide an insight into this phenomenon are outlined below.

As long ago as the 1960s, experimental evidence demonstrated that elevated SCCs in a quarter could protect against coliform infection (Schalm *et al.*, 1964). More recently Shuster *et al.* (1996) experimentally inoculated 12 cows with *E. coli* to induce mastitis; six cows were infected just after calving and six in mid-lactation with the intention of examining why post-parturient cows are more susceptible to *E. coli* mastitis than those later in lactation. It was found that the post-parturient cows did get a more severe mastitis but that it was not due to a slower speed of leukocyte recruitment. In fact post-parturient cows recruited leukocytes faster and to a greater level. The authors did find a lower initial cell count in the cows that had recently calved and suggested that this may have been the reason for the severe mastitis. They attributed the difference in severity of mastitis to an inability of post-parturient cows to control bacterial growth in the first few hours after inoculation, before leukocyte recruitment began. Bacterial numbers escalated greatly in this

Table III
Mastitis Isolates from Low and High BMSCC Herds
(Erskine *et al.*, 1988)

	<i>High</i>	<i>Low</i>
BMSCC	>700 000	<150 000
Mastitis Incidence (/100 cows/year)	35	51
Isolate (% of total)		
<i>Streptococcus agalactiae</i>	41.5	0.0
<i>Staphylococcus aureus</i>	18.3	2.2
Other Streptococci	12.6	12.3
Coliforms	8.0	43.5
No Isolate	8.8	28.6

time and the result was a more severe mastitis. Mid-lactation cows that started with more cells in the milk were apparently able to control early bacterial growth to a greater extent. One cow that did have a particularly slow speed of leukocyte recruitment to the udder, however, became the most severely affected of all (Shuster *et al.*, 1996). This agreed with earlier findings (Hill, 1981), that a slow PMN response can be important in dictating the severity of clinical disease. These findings were supported by a meta-analysis of several *E. coli* challenge studies by Van Werven *et al.* (1997), which found that cows with low blood and milk leukocyte counts also recruited cells more slowly on intramammary challenge and subsequently developed more severe disease.

Schukken *et al.* (1999) found that cows with a higher SCC were less likely to become chronically infected when experimentally challenged with *S. aureus*. Cows that succumbed to infection had a mean cell count of 36 000 cells/ml whereas cows that were able to eliminate infection had a pre-challenge somatic cell count of 122 000 cells/ml, demonstrating that increased susceptibility to infection only appears to occur at 'very' low SCCs.

It would appear that both initial numbers and speed of migration of leukocytes are important in a cow's defence against clinical mastitis, and that these two phenomena appear to be correlated. However, other factors should not be overlooked—nutritional status, in particular poor vitamin E and selenium status (Smith & Hogan, 1993) as well as negative energy balance (Suriyasathaporn *et al.*, 1999) have been demonstrated to influence the speed of neutrophil recruitment and migration.

The SCC story has yet to be fully elucidated and more research is still required. It will probably not be as simple as the number of cells present in milk—the type/sub-type of cell, its ability to function and the speed of its recruitment to the udder will all almost certainly play a role.

The role of the minor pathogens

Studies in both the Netherlands and the UK have found that in low BMSCC herds (<150 000/ml) post milking teat disinfection can increase the risk of *E. coli* mastitis (Schukken *et al.*, 1991; Lam *et al.*, 1997; Peeler *et al.*, 2000). These authors have postulated that this effect may be through the removal of minor pathogens (e.g. *Corynebacterium* spp. and Coagulase negative Staphylococci) from the mammary gland and, therefore, the removal of the protective effect these bacteria may provide. Numerous studies have attempted to investigate the potential protective

properties of *Corynebacterium bovis* but these studies have identified both protective (Pankey *et al.*, 1985; Lam *et al.*, 1997) and detrimental (Brooks *et al.*, 1983; Pankey *et al.*, 1985; Hogan *et al.*, 1988) effects. A more recent study has demonstrated that quarters acquiring a new intramammary infection with a *Corynebacterium* spp. during the dry period were significantly protected from subsequent infection with a major mastitis pathogen whereas quarters infected with a *Corynebacterium* spp. at drying off were at a significantly increased risk of acquiring a new intramammary infection (unpublished data). These findings may go some way to explaining the contradictory findings of earlier studies as it could be that quarters infected with a *Corynebacterium* spp. at drying off are inherently susceptible to infection with any pathogen which may then mask the potentially protective effects of infection with this species of bacteria.

The role of minor pathogens might be linked to somatic cell count levels as they may stimulate a small cellular response in the udder, or it may be that their protective role could arise from 'competitive exclusion'. Woodward *et al.* (1987) demonstrated competition between *C. bovis* and other mastitis pathogens in vitro, but further studies are required to investigate this phenomenon. In another study the presence of a bacteriocin known to be inhibitory to *S. aureus* was shown to reduce the incidence of infection during the dry period when used alone and in combination with a dry period internal teat sealant (Ryan *et al.*, 1999; Twomey *et al.*, 2000).

Further evidence for the importance of bacterial interactions within the mammary gland comes from the reports of clinical *E. coli* mastitis following blitz therapy (whole herd antibacterial treatment for removing *Str. agalactiae* infection) (Boyer, 1997; Bradley & Green, 1997; Edmondson, 1997). Boyer (1997) reported an increase in the incidence of environmental mastitis for weeks following blitz therapy with erythromycin. One possible explanation for this observation could be the accidental inoculation of environmental pathogens at the time of infusion, though this would not explain the increased incidence over several weeks. It could be that as the bacteriological and immunological environment within the mammary gland changed following blitz therapy (which would have effectively removed *C. bovis* as well as *Str. agalactiae*), newly acquired or pre-existing infections that would previously have been 'suppressed' may become manifest.

The interactions of different species of bacteria both with each other and with the mammary gland itself have been somewhat overlooked in recent years

in the quest for 'sterile' milk. Study of such interactions is likely to improve our understanding of bovine mastitis and may lead to novel approaches to controlling mastitis that are less reliant on the use of antibiotics.

Persistence of environmental pathogens in the mammary gland

It would seem unlikely that the fall in SCCs alone could account for the dramatic changes seen in mastitis incidence and aetiology. There does not appear to be a linear relationship between SCC and clinical mastitis, although this is recognised at high SCCs. Peeler *et al.* (2000) found a range of between 0.6 and 147.1 cases of clinical mastitis/100 cows/year in their study of herds with BMSCC under 100 000 cells/ml, indicating that low SCC herds can experience both a very low and very high incidence of clinical mastitis. The authors identified a number of risk factors and advantageous management strategies. One factor that this study could not have identified, but that could be influencing the incidence of mastitis on the different farms, is the strain of organism present on each unit and more importantly the relative ability of those strains to cause intramammary infection and persist.

The role of the dry period and importance of recurrence in environmental mastitis

One factor identified by Peeler *et al.* (2000) in their recent UK study was that herds with a mean dry period length of >40 days were at increased risk of clinical mastitis. Classically, the non-lactating mammary gland has been considered refractory to 'coliform' infection (Jones, 1990). However, research in the US, from as early as 1943, has implicated the dry period as being the time of greatest risk for the acquisition of both new gram-negative and gram-positive intramammary infections (IMIs) (Murphy & Hanson, 1943; Eberhart & Buckalew, 1977; Oliver & Mitchell, 1983; Smith *et al.*, 1985; Todhunter *et al.*, 1991), with some 61% of all new IMIs occurring at this time (Todhunter *et al.*, 1991). Experimental studies have demonstrated the ability of such infections to remain quiescent within the udder until calving, subsequently causing clinical mastitis in early lactation (McDonald & Anderson, 1981).

A recent UK study extended this research by using DNA fingerprinting to demonstrate conclusively the persistence of enterobacterial organisms acquired during the dry period, which subsequently caused clinical disease (Bradley & Green, 2000). This study of six well managed low

BMSCC herds demonstrated that over 50% of all enterobacterial clinical mastitis occurring in the first 100 days of lactation arose from infections acquired during the dry period. Although not reported in that paper, one quarter remained persistently infected (as confirmed by DNA fingerprinting) for over 200 days before succumbing to severe per-acute clinical mastitis. Preliminary analysis of the same dataset has demonstrated that over 50% of *Str. uberis* clinical mastitis also arose in quarters that became infected during the dry period (Bradley & Green, 2001b). The clinical importance of the dry period and persistent infection in coliform mastitis epidemiology has recently been confirmed by an intervention study that demonstrated a 50% reduction in clinical coliform mastitis incidence, during the first 100 days of lactation, in cows treated with an antibiotic dry cow therapy with persistent activity against Gram-negative organisms (Bradley & Green, 2001c).

Persistent infection with both *Str. uberis* (Todhunter *et al.*, 1995; Watt, 1997) and *E. coli* (Hill & Shears, 1979; Hogan *et al.*, 1989; Lipman *et al.*, 1995; Lam *et al.*, 1996; Dopfer *et al.*, 1999; Bradley & Green, 2001d) has been reported by a number of authors. Studies in the Netherlands have suggested that 9.1% (Lam *et al.*, 1996) and 4.8% (Dopfer *et al.*, 1999) of clinical *E. coli* mastitis was recurrent when studying herds with BMSCCs of less than 150 000 and 400 000 cells respectively. A more recent study in the UK of clinical mastitis in six low BMSCC herds in Somerset identified *E. coli* as being the most common cause of recurrent clinical mastitis, with 20.5% of all cases being recurrent, as confirmed by DNA fingerprinting (Bradley & Green, 2001d). The recurrent clinical cases on these farms were significantly less severe than single cases and the mean time between recurrent cases was 42.8 days. This study also identified similarities in the DNA fingerprints of *E. coli* strains causing recurrent mastitis on four of the six farms, data which when considered in its entirety is suggestive of a degree of adaptation to the bovine mammary environment (Bradley & Green, 2001d); such adaptations to the bovine environment could for instance be an increased ability to sequester iron, survive with mammary neutrophils or adhere to and invade mammary tissue.

Once significant levels of sub-clinical, persistent *E. coli* infection exist within a herd, the possibility of spread of infection from one cow to another during the milking process needs to be addressed. To date it has been assumed that *E. coli* behaves in an entirely opportunistic manner (Nemeth *et al.*, 1994) and that

environmental strains are equally likely to cause intramammary infection. A recent study of one dairy herd in the UK did not find any correlation between strains of *E. coli* in the environment with either those in the bulk tank or those isolated from clinical and sub-clinical mastitis episodes. However, the predominant strain in the bulk tank was also the predominant strain causing both clinical and sub-clinical mastitis (Bradley *et al.*, 2001). It has previously been assumed that the presence of *E. coli* in milk is a result of environmental contamination (Blowey & Edmondson, 1995); the data collected by Bradley *et al.* (2001) suggested that an udder reservoir, under certain circumstances, may make a significant contribution to the bulk tank. This small study needs to be treated with caution as it is based on just one farm and all studies of this type are fraught with how best to obtain samples representative of the environmental population.

Mechanisms of bacterial persistence within the udder

Any organism causing persistent infection of the mammary gland must have mechanisms to avoid removal by regular milking and to evade the immune system. There has been extensive research of possible strategies adopted by *Str. uberis* and these mechanisms have been discussed in detail elsewhere (Leigh, 1999). In summary, research has demonstrated the ability of *Str. uberis* to resist phagocytosis (Thomas *et al.*, 1994) and intracellular killing by leukocytes (Leigh *et al.*, 1990). Adherence is not thought to be important in the early stages of pathogenesis of *Str. uberis* mastitis though the ability of certain strains to adhere both to extra-cellular matrix (Lammers *et al.*, 2001) and to bovine mammary epithelial cells in the presence of fibronectin (Almeida *et al.*, 1999) may be important in the subsequent development of persistent infection.

In contrast to *Str. uberis* the mechanisms of persistence of *E. coli* in the bovine mammary gland are less well understood. Serum resistance of *E. coli* has previously been associated with organism virulence in the bovine mammary environment (Sanchez-Carlo *et al.*, 1984) and serum resistant strains have been shown experimentally to be capable of surviving for protracted lengths of time in the mammary gland (Hill & Shears, 1979). As early as 1979 researchers demonstrated experimentally the ability of *E. coli* to cause persistent infection in the mammary gland in the absence of a significant immune response, and demonstrated the presence of viable bacteria within neutrophils (Hill *et al.*, 1979). Survival

of bacteria in neutrophils alone is unlikely to be the only mechanism of persistence especially considering the short half life of neutrophils in milk, which would necessitate repeated invasion of fresh neutrophils by the bacteria and hence their exposure to the immune system. More recent research has demonstrated the ability of *E. coli* to adhere to mammary cells, mediated both with and without the presence of fibronectin (Lammers *et al.*, 2001). Researchers in both the Netherlands (Dopfer *et al.*, 2000) and the UK (Bradley *et al.*, 2001) have demonstrated an increase in the ability of 'recurrent' strains of *E. coli* to adhere to and invade the Mac-T tissue culture adapted bovine mammary epithelial cell line. Further research has demonstrated that certain *E. coli* strains were as adherent as *Str. dysgalactiae* and more adherent than *Str. uberis*, though less adherent than *S. aureus* (Dopfer *et al.*, 2001). This same research investigated the mechanisms by which two *E. coli* strains invaded epithelial cells, but although demonstrating the role of cytoskeletal elements in the invasion process, it failed to detect genes encoding proteins characteristic of EPEC strains of *E. coli*. The authors hypothesised the existence of some cytoskeletal mediated uptake reliant on the presence and action of certain phosphokinases by an unknown mechanism (Dopfer *et al.*, 2001).

E. coli is recognized as a highly adaptive organism existing as both a commensal and as a pathogen; its ability to acquire exogenous DNA, and hence virulence genes, is well established (Dozois & Curtiss, 1999): This process could play a role in the emergence of udder adapted strains, such as those possibly identified in a recent UK study (Bradley & Green, 2001d). Many different subsets of *E. coli* have also been demonstrated, such as enterohaemorrhagic, enteroinvasive and enteropathogenic, and it is not unreasonable to expect that another such subset more adapted to the mammary environment may already be present but be, as yet, unidentified.

CURRENT CHALLENGES

Probably the biggest challenge facing the modern dairy industry is the pressure to reduce the use of antibiotics in food producing animals, coupled with the dramatic increase in organic milk production in recent years. Mastitis remains a significant problem in organic dairy herds (Hovi & Roderick, 1999) and the dry period is particularly difficult to manage in the absence of antibiotic dry cow therapy, with significantly more new intramammary infections

occurring in untreated quarters (Berry & Hillerton, 2000).

This pressure on the use of antibiotics, and the apparent lack of progress in mastitis control in recent years, has led to an increase in the use of alternative medicines. There is little or no peer reviewed scientific evidence to support the use of these products and in the absence of such studies it seems difficult to recommend the use of such 'liniments', 'rubs' and nosodes.

One area that has, however, shown promise is the development of both 'internal' and 'external' teat sealants for use during the dry period. Independent studies of a polymer-based external teat sealant (DryFlexTM, Delaval) in the US have demonstrated its ability to reduce the incidence of new intramammary infection during the dry period (Timms, 2001). Although the product is probably best seen as an adjunct to antibiotic therapy rather than as an alternative it may have a role to play in the control of intramammary infections in organic herds and in peri-parturient heifers.

Probably the single biggest drawback of external sealants is their lack of persistence (Hayton & Bradley, 2001). This problem has been overcome with the use of an internal sealant (Teatseal, Cross Vetpharm Group) which, though readily milked out at calving, has been shown to persist in the teat cistern for over 100 days following infusion at drying off (Woolford *et al.*, 1998). Research in both New Zealand (Woolford *et al.*, 1998) and the UK (Huxley *et al.*, 2001) has demonstrated the efficacy of this product in preventing new intramammary infections in quarters uninfected at drying off; and in the future is likely to be the product of choice for prevention of new intramammary infections during the dry period.

FUTURE PROSPECTS

The 'Holy Grail' of mastitis control remains vaccination, and yet despite decades of research no 'truly effective' vaccine is yet commercially available. *E. coli* J5 core antigen vaccines have been widely available in the US for a number of years and one such vaccine has recently become available in Europe (Enviracor, Pharmacia). The efficacy of such vaccines in reducing both the incidence and severity of clinical disease, by ameliorating clinical signs, has been demonstrated by a number of authors (Gonzalez *et al.*, 1989; Hogan *et al.*, 1992a), although the vaccine seems unable to prevent new coliform intramammary infections (Hill, 1991; Hogan *et al.*, 1992a;

Hogan *et al.*, 1992b). Further research focusing on the development of sub-unit *E. coli* vaccines, using antigens such as the iron acquisition receptors FepA and FecA may hold promise in the future (Lin *et al.*, 1998; Lin *et al.*, 1999). Perhaps the most exciting progress in the field of mastitis vaccination has been against *Str. uberis* with the development of a subunit vaccine against the plasminogen activator *pau A* (Leigh *et al.*, 1999). Early studies have shown promise and offer the elusive goal of being able to 'control mastitis without causing mastitis' by engendering an effective immune response without the massive recruitment of neutrophils to the mammary gland with the subsequent effects on milk quality.

One other area of possible future research that may yield novel approaches to the control of bovine mastitis is through a greater understanding of mammary ecology. There are undoubtedly complex interactions between different strains and species of bacteria within the mammary gland, some of which have been outlined earlier in this paper. Studies of these interactions may yield new therapeutic agents or even lead to the development of 'mammary probiotics' that could be utilised during the non-lactating period.

In the shorter term we need to focus our efforts on improving environmental management, perhaps through the employment of novel bedding systems such as sand (Green & Bradley, 2001), and also to approach the management of mastitis in a more holistic manner by ensuring optimal nutrition, minimising stress and encouraging farmers to pay attention to detail.

CONCLUSIONS

Mastitis remains a complex disease and its management is an increasing challenge. The implementation of the Five-Point Plan and similar mastitis control programmes worldwide has led to a dramatic decrease in the incidence and prevalence of disease, though these improvements could be rapidly lost in the absence of continued implementation of these control strategies. Unfortunately much of this improvement in mastitis control to date has been on the back of widespread and unsustainable antibiotic usage. Meanwhile, there has been a concurrent change in the aetiology of mastitis and there is an increasing body of evidence to suggest that the environmental pathogens are more able to adapt to the mammary environment than previously thought. These changes provide an unprecedented opportunity to study the ability of organisms to adapt to

changing environments, and along with the pressure to reduce antibiotic usage will provide new challenges in the future to both the researcher and clinician alike.

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