

PROGRESS IN MILK QUALITY?

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INTRODUCTION

For many decades the UK dairy industry has come to expect an annual decline in the somatic cell count (SCC). However, recent information from National Milk Records (NMR) shows that on recorded farms this trend has gone into reverse.

ADAS MILK LABORATORY RESULTS

Over 30 dairy companies use the ADAS Milk Laboratory to provide them with individual farm bulk milk quality. The SCC information that this provides is representative of the industry and taken from a large range of dairy farms. Since 1994 (de-regulation) the ADAS Milk Laboratory results have shown a steady decline in SCC. From 2000 the SCC have unfortunately began to rise with an acceleration seen.

REASONS FOR INCREASING SCC

There is unlikely to be only one reason for an increasing level on a farm, with a combination of several factors the probable cause. This combination will vary from farm to farm. Time spent by farmers, their advisors or veterinary surgeons in investigating the underlying reasons will pay dividends in correcting the problem.

The trend of increasing SCC was seen prior to the outbreak of FMD, although this subsequently has had a major affect on herd SCC this past 12 months. The restriction in animal movements resulted in "cull" cows remaining on the farm. Rather than these animals being dried off, most were allowed to continue in milk and run with the herd, regardless of the reasons for potential culling. Any cows with high SCC or those chronically affected with recurring episodes of clinical mastitis became a reservoir of infection for the rest of the herd. Contagious mastitis organisms such as *Staphylococcus aureus* are readily spread through the milking machine. Much evidence also indicates that *Streptococcus uberis* is also becoming more persistent and would have spread from infected cows. Many cow houses were also overcrowded.

The lactation length of the majority of the national herd was seriously affected by the lack of AI during the FMD outbreak. Long lactation cows tend to have higher SCC for several reasons, including lack of dilution effects and greater exposure to mastitis causing organisms.

A falling milk price since the late 1990s has seen many dairy producers cutting costs. Unfortunately, many economised on those measures that have a direct affect on mastitis management.

These cuts included reducing the frequency or even stopping post-milking teat disinfection, minimising teat preparation or being selective with dry cow therapy. Stopping such control measures do not usually have an immediate effect, therefore many producers did not associate rising SCC or clinical mastitis with actions taken 3–6 months earlier and so continued with the cut backs.

A reduction in labour has put increasing pressure on those remaining at the front line. Corners have been cut: poor teat disinfection is as bad as none, improper/inconsistent teat preparation increases the risk of environmental mastitis and can adversely affect milk let down.

Milk let down is a particular issue with high throughput parlours where teat preparation is minimal. Extended milking (unit on time) increases the risk of teat damage where the milking machine is not working efficiently. Poor cluster position in any parlour can increase the risk of liner slip, with large parlours this can become a particular issue where there are more cows per side than clusters. Poor milking management also increases the risk of over and under milking – both of which can influence SCC.

Cutting costs by changing liners at longer than recommended intervals or using inappropriate liners will also have affected the efficiency of milk harvesting and contributed to rising SCC. A reduction in parlour service intervals will have resulted in minor problems becoming more significant. Farmers have tried to reduce costs by installing second hand and old equipment, such as pulsators. This can easily result in udder health problems.

Finally, the weather at recent harvests has not been conducive to the provision of ample quantities of dry straw. Practical experience has shown that poor quality and/or insufficient bedding will inevitably lead to dirtier cows and an increased environmental mastitis challenge.

What are the solutions?

MASTITIS MANAGEMENT ACTION PLAN – THE SOLUTION

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INTRODUCTION

The ADAS Milk Laboratory “central testing” results show that after a period of many years of declining herd SCC the trend has now reversed with counts 35% higher than 2 years ago. This is a particularly worrying trend both in terms of farm profitability and due to the serious welfare implications of mastitis (1,2). The reasons for the rise are many and varied, but with correct and prompt management the situation can be reversed.

MASTITIS MAP

In 1998, with funding and support from MAFF (now DEFRA), the Veterinary Laboratories Agency and ADAS produced the Mastitis Management Action Plan (the **Mastitis MAP**). This was published in 1999 by MAFF in the series on Action on Animal Health and Welfare. The **Mastitis MAP** was developed to more effectively control environmental infections but utilised the well proven effectiveness of the NIRD/CVL Five Point Plan for contagious mastitis. The **Mastitis MAP** answers the many calls, including from past British Mastitis Conferences, for a Plan to counter the increasing problems of environmental mastitis as well as to control contagious forms of the disease.

The **Mastitis MAP** is based on the following fundamentals:

Hygienic teat management – it is critical that cows’ teats are kept clean at all times, both at pasture and at housing. A thorough parlour routine can then concentrate on udder stimulation for effective milk let down and shorter unit on times, rather than on excessive time spent cleaning and drying teats. Parlour hygiene is a priority, with correct disinfection of contaminated clusters a priority. Effective post-milking teat disinfection with a licensed product is also vitally important.

Prompt identification and treatment of mastitis - good stockmanship and foremilk, with aids such as in-line milk detectors, are essential for early identification. Milk samples help build a picture of the main causal agents of mastitis, providing greater information for the veterinary surgeon.

Dry cow management and therapy – in addition to correct treatment of existing infections, prevention of new infections by proven control methods and correct dry cow housing are fundamental, but often neglected.

Accurate record keeping – allows for early identification of problem carriers and an essential requirement for:

Culling of chronically infected cows – not a substitute for solving an existing problem but a component of a controlled and comprehensive herd mastitis control policy.

Regular milking machine maintenance and testing – the milking machine is the point of contact with the cow and can influence mastitis by acting as a vector in transferring mastitis causing organisms from cow to cow and by damaging the first line of the udder's defence. Not only must parlours be installed correctly they must be suitably serviced and maintained to ensure they continue to be efficient milk harvesting machines.

Following the **Mastitis MAP** will help ensure falling SCC with a low incidence of clinical mastitis for conventional and organic herds alike.

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AN INVESTIGATION OF THE IMPACT OF APPLICATION TEMPERATURE ON THE ADHERENCE OF AN EXTERNAL TEAT SEALANT

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INTRODUCTION

The dry period is a high-risk time for the acquisition of new intramammary infections (IMI) despite the use of antibiotic dry cow. External teat sealants have been developed to prevent new IMI during the dry period and understanding and controlling factors that effect persistence should lead to enhanced barrier function. External teat sealants are polymer based and may therefore be affected by application temperature. This paper outlines the preliminary results of a study investigating the effect of application temperature on adherence of a commercially available teat sealant (DryFlexTM, DeLaval Ltd.) in two dairy herds, under UK field conditions.

MATERIALS AND METHODS

Two commercial dairy herds were selected on the basis of location, likely compliance with the study protocol and an adequate numbers of cows for inclusion in the study. Mid-dry period cows, at least three weeks from calving, were enrolled in the study. The teat sealant was applied at four temperatures; 2°C, 10°C, 20°C and 30°C. Within each cow one quarter was treated at each of the test temperatures; the quarter treatment at each temperature was rotated counter clockwise on the next cow recruited to the study, thus ensuring that each application temperature was equally distributed between the four quarters. The persistence of the sealant was assessed. Descriptive statistics were evaluated using Statistix and a hierarchical general linear mixed model was fitted using MlwiN.

RESULTS AND DISCUSSION

Forty-nine cows were recruited to the study, each application temperature was applied to 49 teats. Dip persisted for significantly longer when applied at 2°C than at any of the other temperatures (10°C, -1.04 ± 0.38 days; 20°C, -1.16 ± 0.38 days; 30°C, -1.50 ± 0.38 days). Dip persisted longer on one farm (2.58 ± 0.69 days), on teats affected by warts (2.53 ± 0.79 days) and on the right fore quarter (0.66 ± 0.31 days) when compared to other quarters. Significant random variation was identified between cows and between quarters within cow.

This study has demonstrated that application at 2°C will increase the adherence time of DryFlex by more than a day compared to application at UK ambient temperatures. This increase in adherence is likely to be of use to farmers using the dip on a twice-weekly dipping regimen.

AN INVESTIGATION OF THE IMPACT OF DIFFERENT TEAT PREPARATION ROUTINES ON BULK TANK MILK QUALITY

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SUMMARY

The number of bacteria present in milk has implications for milk quality and safety, as well as influencing the final price paid. Bactoscan is now the industry standard for the measurement of bacteria in milk. Five pre-milking teat preparations comprising three pre-milking teat disinfectants, water and dry-wipe only were compared by measuring their effectiveness at reducing bulk tank Bactoscan. The pre-milking teat disinfectants and water were applied using a teat dip cup and the teats were wiped dry with a paper towel. The teat disinfectants and water significantly reduced the Bactoscan reading of milk in comparison to dry-wipe only.

MATERIALS AND METHODS

Five commercial dairy farms in Somerset were recruited to the study, which took place during the winter housing period of 2001-2002. Five different pre-milking teat preparation routines were studied (i) dry wipe only, or treating the teat and then wiping dry following a 30 second contact time with either (ii) water alone, (iii) 2800 ppm available chlorine (Agrisept, Pharmacia Ltd), (iv) 2-4 ppm free iodine (Quartermate, Delaval Ltd) or (v) 500 ppm free iodine (Iodozyme, Delaval Ltd). The treatments were applied by the herdspersons, using a teat dip cup, following the cleaning of any 'grossly' contaminated teats. Each of the five treatment regimes was applied twice on each of the five farms. Each treatment was applied to all of the milkings contributing to a bulk tank collection (i.e. 2 or 4 milkings depending on the frequency of milk collection) and the number of bacteria present in the bulk milk was assessed using the Bactoscan method. Ten percent of the cows in each herd were scored for cleanliness at the beginning and end of the study to assess any concurrent deterioration in the environment. Results were collated and analysed in Statistix (version 7.0).

RESULTS

Dry-wiping resulted in the highest average Bactoscan value of 35.6, and also the highest individual value of 55. The water only preparation resulted in the lowest average Bactoscan value at 22.6, though the lowest Bactoscan result (13) was achieved with Quartermate (Table 1).

Table 1. Average, minimum and maximum values of Bactoscan for each preparation regime

Treatment	Average	Minimum	Maximum
Agrisept	28	21	45
Dry-wipe	35.6	18	55
Iodozyme	22.7	17	32
Quartermate	25.8	13	41
Water	22.6	16	29

There was significant variation in the Bactoscan readings between farms (Table 2). The 'wet' treatments were significantly better than dry wiping and reduced the Bactoscan by between 7.6 to 13 when compared to dry wiping alone.

There was no significant difference in cow cleanliness measured before or after the study.

Table 2. Linear regression model for teat preparation effectiveness. Outcome – Bulk tank Bactoscan count (x10³)

Fixed Terms	n	Coefficient	S.E.	p-value
Constant	10	42.0390	3.17826	
Farm 1	Reference farm			
Farm 2	10	-11.3000	3.34565	0.0016
Farm 3	9	-8.6000	3.34565	0.0140
Farm 4	10	-5.19512	3.44614	0.1395
Farm 5	10	-7.1000	3.34565	0.0401
Dry wipe	Reference treatment			
Quartermate	10	-9.8000	3.34565	0.0056
Iodozyme	9	-12.7951	3.44614	0.0006
Agrisept	10	-7.6000	3.34565	0.0286
Water	10	-13.000	3.34565	0.0004

CONCLUSIONS

Dry wiping does not appear to be the best method for reducing bacterial contamination of milk although is probably the most widely used teat preparation technique during the winter months. The use of a 'wet' step in the teat preparation technique resulted in a significant reduction in Bactoscan. When compared to each other the wet treatments were not significantly different. However, using water applied from a teat dip cup could not be recommended because of the risk of transfer of mastitis pathogens between cows. Pre-milking teat disinfection has the potential additional benefit of decreasing environmental mastitis. Pre-milking teat disinfection should be employed by milk producers wanting to produce milk of the highest standard.

ACKNOWLEDGEMENTS

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THE 'PINT' PLAN - Managing Dry Cows to Maximise Milk Quality

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INTRODUCTION

The importance of the dry period in mastitis epidemiology has been highlighted in the poster 'The Dry Period and Mastitis in Dairy Cows'. This abstract describes an approach to the management of dry cows designed to maximise milk quality and minimize mastitis in the subsequent lactation. The aim of the plan is to focus on the key areas and decisions that need to be made just before and during the dry period and it could form the basis of the dry cow management strategy of a herd health plan.

PREPARE

- Assess and adjust condition score from 100 days prior to drying off (aim for C.S. 2.5 - 3.0 at drying off)
- Reduce production in high yielding cows in the week prior to drying off (by reducing the plane of nutrition)
- Analyse health records to select cows appropriate for different treatments or culling
- Clean and prepare teats properly prior to infusing dry cow therapy (Clean, Pre-dip, spirit swab x2 - infuse with care!!) - This is the biggest investment you make in udder health!

ENVIRONMENT

Keep it COMFY, CLEAN, DRY and WELL VENTILATED

Bedding materials:

- ensure quality (*e.g.* avoid damp straw)
- consider inorganic bedding (*e.g.* sand)

Location:

- fly avoidance
- access and observation

Feed

- ensure adequate access to feed

Manage as well as your lactating cows!

NUTRITION

Avoid fatty liver, ketosis and milk fever and improve milk yield, milk constituent quality, mastitis resistance by ensuring adequate

Energy:

by adding lactating cow forages and an energy dense component such as wheat in the last 3 weeks of the dry period (*NB* avoid lactating cow cake).

Undegradable Protein:

e.g. consider adding bypass soya

Fibre:

by adding at least 1 kg long chop roughage

Vitamins and Minerals:

by ensuring appropriate levels of vitamins and minerals are included in the diet. Vitamin E and selenium in particular play an important role in maintaining udder health.

TREATMENT

*Use appropriate dry cow therapy for **individual cows**, in consultation with your vet, on the basis of:*

Farm History *e.g.* mastitis type and BMSCC

Season *e.g.* summer mastitis

Cow

High SCC (>200,000 = probably infected)
select antibiotic DCT to cure existing infections
target activity at staphylococci and streptococci

Low SCC (<200,000 = probably uninfected)
select DCT to prevent new infections
consider the use of an internal teat sealant
target activity at coliforms and environmental streptococci

Consider other treatments to reduce the risk of mastitis

- Fly control
- External teat sealants
- Vaccination

THE DRY PERIOD AND MASTITIS IN DAIRY COWS

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Recent research has shown that bacteria, particularly *Escherichia coli* and *Streptococcus uberis*, commonly invade the udder during the dry period. In a published UK study, 20% cows had at least one quarter infected with a major pathogen despite use of dry cow therapy. The chance of infection increases with age of cow. Most (>90%) bacteria found in the dry period were new infections after drying off and not carried over from the last lactation.

This research also found that quarters infected with bacteria are much more likely to get mastitis after calving – in fact 6-27 times more likely! In some herds, over half of all mastitis comes from the dry period.

Survival analysis indicated that mastitis from infections in the dry period happens at a faster rate after calving than other mastitis. Approximately 60% occurred in the first 2 weeks of lactation. Therefore the *pattern* of mastitis on a unit can help a veterinary surgeon to determine the importance of the dry period on mastitis on that farm.

We now recommend using a planned approach to dry period management to reduce dry period infections and mastitis after calving. We have called this the “PINT” Plan and it is described in the associated poster at this conference:

“THE PINT PLAN - MANAGING DRY COWS TO MAXIMISE MILK QUALITY”

PRACTICAL USAGE OF AN INTERNAL TEAT SEALER

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INTRODUCTION

Teat sealers (either internal or external) are designed to prevent new infections during the dry period by separating the mammary gland from the environment. They have no antibacterial properties and therefore are only appropriate for quarters that are not infected at drying off. Identifying infected quarters and treating them with antibiotic dry cow therapy will remain a fundamental part of any strategy for controlling mastitis.

Internal teat sealers containing bismuth subnitrate were originally developed in the 1970s by Meaney (4). In the late 1990s a product containing 65% bismuth subnitrate in a paraffin base was launched in New Zealand. It was demonstrated to be as good as antibiotic dry cow therapy at preventing new intramammary infections (IMI) during the dry period (5). Recently the same internal teat sealer was studied under UK field conditions and shown to be better than the market leading antibiotic dry cow therapy at preventing new IMI during the dry period (3). The product studied has recently been launched onto the UK market as OrbeSeal® (Pfizer Animal Health, Sandwich, UK).

PRACTICAL USAGE

Selection of Uninfected Cows

Quarter level bacteriology remains too expensive for routinely identifying infected quarters in a commercial situation. Currently the most appropriate and cost effective method is the use of historical mastitis and somatic cell count (SCC) data. A cow level SCC of 200,000 cells/ml has previously been suggested as a level above which at least one quarter is likely to be infected (2). Recently the last three cow level SCC below 200,000 cells/ml was shown to have the highest sensitivity for identifying quarters infected with Gram-positive organism at drying off, compared to either the last count only or a geometric mean of the last three counts below 200,000 cells/ml (1).

The poster also discusses the potential for using other diagnostic tools such as milk conductivity and acute phase protein levels to identify infected quarters in the future.

Infusion Technique

Internal teat sealers have no antibacterial properties; careful aseptic infusion technique is an important aspect of their use in the field to prevent quarters becoming infected during the infusion process. The poster

describes an appropriate technique for on farm use, the technique is briefly described below.

The viscosity of the internal teat sealer discussed here is affected by temperature; if the product is cold, it is more difficult to infuse. It is a sensible recommendation that the sealer should be stored at room temperature prior to infusion and not left to cool to ambient environmental temperatures especially during the colder parts of winter. To avoid the risk of contamination prior to infusion, the sealer must not be warmed in water.

On-farm technique for the aseptic infusion of an internal teat sealer

- Wear a new pair of clean disposable gloves
- If necessary, clean grossly contaminated teats and udders (ideally with a rapid acting pre-dip) and dry completely with disposable paper towel
- Strip foremilk
- Gently scrub the teat ends of all four teats with a disposable spirit swab until clean. Use a new swab for each teat. Clean the teats furthest away from you first to avoid contaminating cleaned teats.
- Remove the cap from the syringe containing the teat sealer being careful not to touch the tip.
- Infuse the teat sealer into the teat as you would an antibiotic dry cow therapy. DO NOT MASSAGE THE TEAT SEALER UP INTO THE QUARTER. Infuse the quarters closest to you first to avoid contaminating cleaned teats prior to infusion.
- Apply an appropriate post milking teat disinfectant and confine the treated cows to a loafing yard for at least 30 minutes to allow the teat canal to close.

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C. bovis – FRIEND OR FOE?

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INTRODUCTION

Corynebacterium bovis is a member of a “lipophilic” sub-grouping (10 species) within the genus *Corynebacterium* whose growth is enhanced by the addition of free fatty acid (1% v/v Tween 80) to the growth medium.

Intramammary infection (IMI) with *C. bovis* is associated with a significant elevation in SCC; the literature suggests that compared to culture negative quarters, the increase is in the order of 50,000 cells/ml for both naturally acquired and artificially induced infections. Previous work has suggested that quarters infected with *C. bovis* are significantly less likely to become infected with other more pathogenic organism i.e. *C. bovis* protects quarters from infection. Conversely some authors have demonstrated the opposite affect i.e. quarters infected with *C. bovis* are more likely to become infected with other pathogens.

METHODS

All quarters of all cows in a 500 cow, 16 farm study in the SW England were sampled for bacteriology and SCC at drying off, calving and seven to 14 days after calving (2). Cows were eligible for inclusion in the study if they had no cases of clinical mastitis and all cow level somatic cell counts (SCC) less than 200,000 cells/ml during the previous lactation.

Seven hundred and sixty two lipophilic *Corynebacterium* species were isolated during the study. *C. bovis* was differentiated from other lipophilic *Corynebacterium* species by endonuclease restriction analysis of the 16S rRNA gene sequence (1). The impact of *C. bovis* infection on SCC and rate of infection with other more pathogenic organisms was assessed using univariate and multivariate statistical techniques.

PROVISIONAL RESULTS

Quarters infected with *C. bovis* had significantly higher SCC than quarters that were bacteriologically negative (Drying off: 457,100 *cf.* 173,800 cells/ml; Calving 933,300 *cf.* 371,500 cells/ml; 7-14 days after calving 208,900 *cf.* 45,700 cells/ml).

Compared to quarters not infected with *C. bovis*, quarters infected with *C. bovis* were significantly less likely to be concurrently infected with coagulase positive staphylococci, *Enterococcus* spp. and major pathogens at drying off;

all Enterobacteriaceae and major pathogens at calving; and coagulase positive Staphylococci and major pathogens seven to 14 days after calving.

Quarters that retained a *C. bovis* infection during the dry period were significantly less likely to acquire a new dry period IMI caused by a major pathogen, compared to quarters that were not infected at either sampling time point.

DISCUSSION

This is the first study to demonstrate that quarters which retain a *C. bovis* infection are protected from subsequent infection with a major pathogen during the dry period. Other authors have demonstrated that the same affect is seen during lactation.

Quarters infected with *C. bovis* had higher SCC than bacteriologically negative quarters at all sampling time points; this is in agreement with previous finding. The elevation in SCC in this study was higher then that described previously probably because samples were collected after last milking at drying off, immediately after calving and one week after calving; other workers have demonstrated that SCC are higher than during “mid lactation” at all of these times.

IMI with *C. bovis* undoubtedly elevates the SCC of infected quarters, which considering the financial pressures on producers to produce bulk milk within certain SCC thresholds e.g. <150,000 cells/ml, must be considered a disadvantage. However it also appears that quarters infected with *C. bovis* are protected from IMI with other pathogens. Subclinical IMI with major pathogens elevates the SCC significantly more than IMI with *C. bovis* (3). By accepting a small increase in SCC due to *C. bovis*, producers may be “protecting” themselves against substantially higher SCC caused by an increased prevalence of subclinical major pathogen IMI. Further work is needed to investigate this potentially interesting interrelationship.

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EXPERIMENTALLY INDUCED TEAT STENOSIS IN DAIRY EWES: CLINICAL, PATHOLOGICAL AND ULTRASONOGRAPHIC FEATURES

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A strain of *Staphylococcus chromogenes* was inoculated into the teat cistern of ewes and the effects of infection were studied. Mastitis, assessed by clinical, cytological and bacteriological findings, was evident 4 days after challenge. Teat inflammation and consequently, teat stenosis were also prominent consequences of the challenge. Initially, the inoculated teats were swollen and warm. At a later stage, a hard structure was palpated running lengthwise inside the teat; a thick ring was also palpated above the tip of the teat, whilst expression of milk was difficult. Ultrasonographically, a hyperechoic line under the mucosa of the teat cistern was observed. At *post-mortem* examination, the wall of the duct of the inoculated teats was found to be thicker than normal. Histopathological features included extensive fibrous tissue proliferation in the subcutaneous tissues and leucocytic infiltration, especially under the mucosa of the teat cistern. *S. chromogenes* was isolated from scrapings from the teat duct and teat cistern, as well as from the mammary tissue samples obtained, from the inoculated ewes.

ACTIVATION OF THE KININ-SYSTEM IN BOVINE MILK: A POSSIBLE MARKER FOR BACTERIAL PATHOGENS OF MASTITIS

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INTRODUCTION

Bradykinin is the most important of the kinins, a family of small powerfully vasoactive peptides. The kinin peptides produce vasodilatation, oedema and pain, and are amongst the most potent pro-inflammatory agents. They are formed from plasma proteins (kininogens) by two families of specific serine proteinases, the kallikreins. Plasma-kallikreins are carried as pre-active enzymes in the blood, activated by injury, infection or immune reactions. Tissue, or glandular kallikreins are found in many tissues, especially those having a secretory function, although their precise role remains to be determined. The formation of bradykinin-like activity in milk was first reported by Guth (3) and subsequently, bradykinin was shown to be present in bulk milk used for human consumption (4). Bovine mammary homogenates have also been shown to contain a 'glandular' kallikrein (5).

Bovine mastitis is accompanied by marked oedema and tenderness in the affected quarters of the udder and bradykinin is one of the most potent known mediators of pain and oedema. However, until the present ongoing study, no attempt had been made to measure kinin-system components in milk, nor to determine how they varied in the presence of disease.

BRADYKININ IN MILK FROM MASTITIC UDDERS

We recently reported that bradykinin levels were raised in milk from mastitic quarters in direct relation to the mastitis severity (2). These findings led us to suggest, at a recent British Mastitis Conference (8), that the increase in milk bradykinin may be important in the mediation of acute inflammatory symptoms in bovine mastitis. However, the activation of bradykinin release in milk during mastitis did not appear to be merely secondary to onset of the inflammatory process, which is the case in other body tissues (1) and at that time, the mechanism of the bradykinin increase was very unclear. When one or more quarters in an udder were diagnosed as mastitic, the bradykinin increase was found in milk from both the inflamed and associated 'non-involved' quarters. The raised kinin levels did not require there to be raised somatic cell counts in the same samples nor were they secondary to changes in blood levels of kinin. However, in both naturally occurring and experimental mastitis, the increase in bradykinin was associated with the presence in milk of pathogens such as *Staphylococcus aureus* (2).

CURRENT FINDINGS

We have since reported that the healthy bovine udder is capable of secreting kallikrein and kininogen directly (6). Normal healthy bovine milk contains both tissue and plasma-kallikrein and the kallikrein activity increases in the presence of mastitis (7). The formation of bradykinin in bovine milk can be activated directly, *in vitro*, by both the Gram-negative *Escherichia coli* and the Gram-positive *S. aureus* (9). It is now clear that both *E. coli* and *S. aureus* can directly activate both plasma and tissue kallikreins present in milk (10) although probably by different biochemical mechanisms. Current studies will determine whether the activation of the kinin system of milk can usefully be added to the growing number of potential markers for the presence of bacterial pathogens of mastitis.

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PREVALENCE AND TREATMENT OF PERSISTENT *STREPTOCOCCUS UBERIS* INTRAMAMMARY INFECTIONS

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INTRODUCTION

Dairy farmers in the UK are experiencing an increasing problem with *Streptococcus uberis* mastitis cases that respond poorly to conventional treatment. A study was therefore undertaken to investigate persistent *S. uberis* intramammary infections and to trial a novel approach to treatment.

MATERIALS AND METHODS

The response to conventional treatment of *S. uberis* intramammary infection was monitored. Samples were taken for bacteriology and individual quarter somatic cell counting (IQSCC) seven, 14 and 21 days post-treatment. Bacterial identification was confirmed using API 20 Strep (bioMerieux) and restriction endonuclease fingerprinting (REF) was used to strain type *S. uberis* (1). An extended antibiotic treatment regimen was used on the unresponsive cases of *S. uberis* using either three cyclical treatments of procaine penicillin with dihydrostreptomycin or cefquinome intramammary preparations, following the data sheet treatment and milk withdrawal protocol. Treatment monitoring was then undertaken using bacteriology and IQSCC at seven, 14 and 21 days post-extended treatment. Odds ratios were used to compare categories of IQSCC with the bacteriological response.

RESULTS

Milk samples (n=2,257) were submitted from clinical cases of mastitis from 1,657 cows on 130 farms in Devon, England. The farm incidences of mastitis ranged from 1 to 73 cases/100 cows/year with a median of 13 cases /100 cows/year. The most prevalent bacteria isolated were *S. uberis* (37%), enterobacteriaceae (23%) and coagulase negative Staphylococcus spp. (10%). Unresponsive cases were defined as those in which any of the three post-treatment samples yielded three or more colonies of *S. uberis*. From the 832 cases of *S. uberis* that were identified, 258 were re-assessed and re-sampled post-treatment. Based on the criteria of one positive isolate at any one of the three post-treatment time points, 51% of *S. uberis* infections were shown to be unresponsive to conventional treatment. The odds of responding to conventional treatment in cases that had an IQSCC <400,000 cells/ml were nine times greater than in cases with IQSCC >400,000 cells/ml at day seven after treatment. Cases that showed a decrease in

IQSCC from day seven to day 14, and from day 14 to 21 had almost four and three times greater odds of responding to treatment, respectively compared to cases that showed an increase in IQSCC between any of the time points.

Using the API 20 Strep System the proportion of cases correctly identified using cultural characteristics was 96%. Restriction endonuclease fingerprinting was performed on 75 selected samples taken from 32 cows. Seven were samples from individual cows that responded to conventional treatment and the other 68 were from 25 cows that were hypothesised to have persistent infections. Ninety six percent of the unresponsive cases investigated using strain typing were shown to be persistent infections.

From the 126 cases of *S. uberis* that were shown to be unresponsive to conventional treatment, 75 proceeded to extended antibiotic therapy. Based on the same criteria for persistence as before, 45% of *S. uberis* infections were shown to be unresponsive to extended antibiotic treatment. The odds of responding to extended antibiotic treatment in cases that had an IQSCC <400,000 cells/ml and cases with IQSCC between 400,000 – 1,000,000 cells/ml on day seven were 200 and 30 times greater respectively compared to cases with IQSCC >1,000,000 cells/ml.

DISCUSSION

The importance of *S. uberis* as a cause of clinical mastitis was confirmed and 51% of cases were shown to be unresponsive to conventional treatment. Using strain typing it was shown that almost all these unresponsive cases were persistent infections rather than re-infection with a different strain. Extended antibiotic therapy resulted in a response rate of 55%, which was similar to the response rate for conventional treatment (49%). Follow up samples from 69% of cases given conventional treatment were not monitored due to the cow being culled, dried-off or not sampled for other reasons.

CONCLUSIONS

- *S. uberis* is an important pathogen in clinical mastitis
- IQSCC is a useful indicator as to the likelihood that a case of clinical mastitis has responded to treatment and could be used as a predictor of response to treatment
- Extended antibiotic treatment may be a valuable method of curing persistent infections due to *S. uberis*

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EFFICACY OF ORBENIN LA AGAINST INTRAMAMMARY INFECTIONS WITH *STAPHYLOCOCCUS AUREUS* - A SMALL FIELD TRIAL

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PROTOCOL

Selection - 50 quarters infected with *Staphylococcus aureus* were selected for "label" treatment with Orbenin LA (Pfizer Animal Health, UK).

- The majority (37/50) were high somatic cell count (SCC) cows.
- The high SCC cows were sampled twice, one week interval, to confirm the infection. Only cows positive for *S. aureus* on both samples were included on the trial. Individual quarter SCC were measured at the time of the second sample.
- In some high prevalence herds clinical cases were treated with Orbenin LA and confirmed as *S. aureus* infections retrospectively. Only *S. aureus* cases were included on the trial.
- Clinical cases were treated after the first sample and no cell count at the time of sampling was available.

Treatment – "label" treatment as per data sheet.

- 3 x Orbenin LA (200 mg cloxacillin) at 48 hour interval

Post-treatment Sampling

- Bacteriology & SCC at 7, 14 & 21 days after last treatment

Successful treatment was based on a failure to culture S. aureus from any of 7, 14 or 21 day post treatment sample.

Further evaluation of success was based on evaluation of post-treatment individual quarter SCC.

Based on bacteriological examination of quarter samples 7, 14 & 21 days after the last treatment Orbenin LA gave an overall cure rate of 50%.

DISCUSSION

There appeared to be a farm cure rate effect that might be related to :-

- Strain variation
- Speed of identification of infection
- Duration of infection
- Type of infection treated (clinical or high SCC)

Assessment of treatment success of *S. aureus* intramammary infection in a herd with even a low prevalence of *S. aureus* cows is open to interpretation.

Is there an optimum time to assess the efficacy of treatment?

If sampled too early after treatment there is an increased possibility of false cures. The infection may have been suppressed but not eliminated.

If sampled too late after treatment there is a chance that a positive culture after earlier negative cultures could be re-infection.

Treatment of intramammary *S. aureus* infections during lactation is known to be difficult and success rates are often little better than 50%. Treatment during the dry period improves success rates and culling is the ultimate treatment. Waiting until drying off to treat known infected cows can allow a significant spread of infection within a herd even with good milking routine. Culling all *S. aureus* infected high SCC cows as soon as they are identified is not economically justified and can rarely be tolerated. Treatment of high SCC cows found to be infected with *S. aureus* during lactation offers an alternative to culling and has a place in limiting the spread of infection within the herd. Even if suppression of infection is all that is achieved it is still economically viable to help manage a herd *S. aureus* problem. Some apparent failures of treatment when infection is found some months later, after negative culture post-treatment checks, may well be re-infection rather than just suppression of infection.

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BOVINE STAPHYLOCOCCAL MASTITIS

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Staphylococcus aureus causes approximately 10 clinical cases of mastitis/100 cows/year in Britain; however, the vast majority of infections are sub clinical and therefore not detected by routine examination of the udder. As a consequence, testing of milk is required to determine if the udder is infected. This has implications for cure rates since some *S. aureus* infections are highly refractory to antibiotic therapy.

Early detection of infection has been shown to improve cure rates. We present the early results of a technique employing enzymatic clearance of milk, which allows rapid detection and enumeration of staphylococci in milk using fluorescent technology. The aim of this research is to facilitate early treatment and improve cure rates for *S. aureus* mastitis, both clinical and subclinical.

Further to this research, information from an organic herd is being collected from both milk, and the dairy cow environment. Individual quarter milk samples are being collected every four weeks from 21 first lactation heifers and 17 multiparous cows, to determine their infection status. Individual quarter somatic cell counts (IQSCC) are also recorded for the heifers, and composite SCC for the cows. Heifer body sites (teats, vagina and mouth/nose) have been sampled, in addition to feed and water troughs, cattle handling facilities, cubicle partitions, various areas of the milking machine and parlour, feed and bedding itself, milking personnel, non-bovine animals and the air. All are tested for the presence of *S. aureus*. To date, *S. aureus* has been isolated from milk, clusters, personnel, non-bovine animals and the environment.

These isolates, and others from international collections, will be examined using multi-locus sequence typing (MLST) to determine the diversity of the isolates collected. MLST has advantages over other techniques because it can produce high levels of discrimination, it is transferable between laboratories and it is ideal for global epidemiology. This will then allow us to answer:

- i. what are the sources of intramammary *S. aureus*?
- and,
- ii. does strain variation affect the severity of disease?

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AN INVESTIGATION INTO THE EFFECTIVENESS OF PRE-MILKING TEAT-CLEANING REGIMES

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A new two-year project is starting. The aims are:

- To study and evaluate the effectiveness of current pre-milking teat-cleaning regimes, using controlled studies
- To produce practical guidelines to distributed to dairy farmers on completion of the project

As part of the study, we will be sending a questionnaire to milk producers. We are calling for participants to be involved in this process.

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EFFECT OF TWO SELECTIVE DRY COW STRATEGIES ON QUARTER INFECTION STATUS AT CALVING

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Dry cow therapy as a prophylactic control measure is usually carried out on the whole herd in the UK but it is recommended only as a therapeutic measure for individual quarters within a cow in some countries.

Within the cow intra mammary infection status may be determined at the cow or quarter level. Using quarter as the unit of infection assumes that each quarter within a cow has a risk of being infected independently of the other quarters. Probably the quarters within a cow are more alike in susceptibility to clinical mastitis in lactation than would be expected based on independence of quarters and this appears to be the case in the lactating cow. However, this has not been tested for infections occurring in the dry period.

Knowledge of whether quarter independence of intra mammary infection occurs over the dry period, could influence how dry cow therapy is best applied as either a prophylactic or therapeutic measure.

The frequencies of new infections of individual quarters have been determined in large field studies on commercial dairy farms. Cows were allocated at random, at drying off to either a treated or an untreated group. Treatment was either an antibiotic dry cow product or an internal teat sealant Orbeseal[®], depending on the trial. All quarters of cows at drying off were either uninfected or infected with *Corynebacterium* spp. or coagulase negative staphylococci (CNS). The quarter was determined as infected if any of *Staphylococcus aureus*, *Arcanobacterium pyogenes*, any streptococci or any coliform bacteria were detected at calving. Treatments in both trials were allocated at the cow level but infection status at calving was determined at the quarter level.

Examination for the clustering or interdependence of infection at calving among quarters was by:-

- Comparing the distribution of the number of cows with different numbers of quarters infected to the number of cows expected within each classification, based upon the prevalence of infection and a binomial probability distribution
- Testing the deviation from binomial probability distribution using a goodness-of-fit test (Proc Freq, SAS Version 8.1)
- Multiple regression modelling to examine for the effect of treatment on infection status at calving, controlling for quarter

interdependence, including a variance inflation factor (Proc Genmod, SAS Version 8.1).

Interdependence between quarters was demonstrated with respect to infection status at calving overall and for the untreated groups in both trials. However, there was no evidence for interdependence among quarters receiving either antibiotic or Orbeseal® treatment. Being infected at drying off increased the probability of infection at calving.

The use of either an antibiotic or an Orbeseal® dry cow strategy:

- reduced the overall incidence of a new intra mammary infection and
- reduced the risk of cows acquiring an infection in more than one quarter

Comparable results were obtained with the dry cow antibiotic and the Orbeseal® strategies.

Table. Final model of infection status at calving in the trial of two selective dry cow strategies. Models obtained using backward stepwise logistic regression (Proc Genmod, SAS Version 8.1). Variance inflation factors included for both trials ($\beta = 1.9484$ for antibiotic trial, $\beta = 1.5855$ for Orbeseal® trial)

Trial	Variable	Coefficient (β)	Estimate (OR)	Wald 95% Confidence Limits	p-value
Antibiotic	Intercept	-2.7376	-	-	<0.0001
	Treatment ^a	-1.1957	0.302	0.101 – 0.907	0.0329
	Parity ^b	0.7666	2.15	0.731 – 6.34	0.1643
	Infected at drying off:^c				
	Corynebacteria	0.6955	2.00	0.642 – 6.26	0.2311
	CNS	0.8102	2.25	0.522 – 9.68	0.2767
Orbeseal®	Intercept	-2.4755	-	-	<0.0001
	Treatment ^a	-1.2058	0.299	0.106 – 0.844	0.0226
	Infected at drying off:^c				
	Corynebacteria	1.0681	2.91	1.06 – 7.99	0.0381
	CNS	1.1053	3.02	0.799 – 11.4	0.1034

^a – treated cows relative to untreated cows

^b – parity >2 relative to parity <=2

^c – cows infected at drying off relative to cows uninfected at drying off

VARIATION IN THE SURFACE OF A MILKING LINER AFTER 4000 MILKINGS

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The surface of the milking liner has been examined as part of a study of its deterioration with age (1). DeLaval 960000-01 liners were aged naturally by milking a herd of 230 dairy cows twice daily. All operating conditions, milking machine and wash methods, were monitored to be within the experimental protocol and industry norms. The study lasted approximately 6000 milkings over 7 months. The internal surface of liners was examined at different ages. The surface, as revealed by scanning electron microscopy (SEM) and X-ray dispersive spectrometry (EDAX) analysis of elements present on the surface of liners after 4000 milkings, is reported.

The bulk composition of the rubber did not change significantly but the liner surface adsorbed fatty acid esters or fatty acids. Some evidence of protein on the surface was obtained too. Although SEM showed that the new liner has a clean and smooth internal surface, the inner surface of the liner barrel developed a crazed appearance with a layer of finely structured material present after approximately 1500-milkings. These changes were greatest in the area near the top of the liner that was in contact with the teat.

EDAX showed that the surface was covered with inorganic matter, a deposition of calcium and phosphate based material largely laid down 8-10 cm from the top of the liner, where the liner barrel folded around the teat during liner closure. Little calcium was deposited elsewhere on the liner.

The calcium may have come from wash water or milk. There was little chloride in the surface layer, and carbonate could not be determined. Because the ratio of calcium to phosphorus was approximately unity, this suggests that milk was the source of the salt deposit.

Generally there was little evidence of chlorine damage to the liner with increasing age. The final rinse waters of the wash cycle never showed use of excessive chlorine. This suggests that the roughness of liners that is detectable with age is the deposition of salts. Whilst there may be a greater risk in hard-water areas, the likelihood is that the calcium and phosphorus come from milk. The coating of the liner may affect its mechanical properties. These are described elsewhere (1). Feeling the roughness of the inner barrel surface of the liner using a finger is a good first indicator of an aged liner.

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