AN EPIDEMIOLOGICAL STUDY OF BOVINE TOXIC MASTITIS

Fraser D. Menzies, Alan W. Gordon and Stewart H. McBride
Department of Agriculture and Rural Development (DARD), Veterinary Sciences Division, Stoney Road, Stormont, Belfast, Northern Ireland BT4 3SD
Email: fraser.menzies@dardni.gov.uk

SUMMARY

A study of acute and peracute toxic mastitis in Northern Ireland obtained information on a representative sample of 264 cases. This paper summarises the clinical and laboratory findings from these cases as well as detailing the main prognostic indicators and individual cow risk factors for the occurrence of toxic mastitis. The majority of cases (84%) occurred during the winter housing period with March being the month of peak incidence (30%). Sixty per cent of cases occurred within one month of calving (29% within four days of calving). Lethargy (92%), discoloured milk (90%) and anorexia (72%) were the main presenting signs; clinical dehydration (44%) and severe pyrexia (18%) were relatively common findings. *Escherichia coli* was isolated in pure culture from half of the milk samples obtained from cases. High plasma creatinine (42%), high plasma urea (31%) and leucopenia (56%) were observed in blood samples obtained from cases.

A case fatality rate of 14% was observed and a further 21% were culled early because of the condition. Recumbency at the time of the first veterinary examination was the main risk indicator associated with an increased case fatality rate while high plasma creatinine levels were also associated with an increased chance of dying from the condition. Risk factors associated with an increased risk of occurrence of toxic mastitis due to *E. coli* were the receipt of parenteral calcium at calving and receiving assistance at calving.

INTRODUCTION

A bovine mortality survey carried out in 1992, identified coliform mastitis as the single most important cause of death in dairy cows (13). The condition was responsible for one in every eight dairy cow deaths, which were seen by a veterinary surgeon.

Given the importance of the condition, DARD's Veterinary Sciences Division carried out an epidemiological study on bovine toxic mastitis (coliform mastitis is the most common type of toxic mastitis).

MATERIALS & METHODS

Individual veterinary surgeons, from a representative systematic sample of 20 farm animal veterinary practices from throughout Northern Ireland, were asked to participate in the study. Sampling kits were supplied, and each kit contained a questionnaire requesting details on cases of acute and peracute toxic mastitis encountered by the veterinary surgeons in the course of their work as well as equipment for collection of blood and milk samples. The milk samples were subjected to routine culture, and antibiotic sensitivity testing was carried out on bacterial isolates. Routine haematology and some biochemical tests (plasma urea, creatinine, haptoglobin) were performed on any blood samples received. The information from the questionnaires, along with any laboratory results, was entered onto a database to facilitate data analyses.

The herd keepers identified by the veterinary surgeons were subsequently contacted and asked to complete questionnaires on the fate of the affected cow and on the next cow to calve. A follow-up telephone call was made to herd keepers who did not return the questionnaires to find out how the mastitis case had progressed.

The information received was validated by a number of cross-checks before any statistical analyses were carried out. The data were initially summarised to provide an overview of the condition in relation to its temporal occurrence, and the clinical and laboratory findings associated with cases of toxic mastitis. The association between the various clinical and laboratory findings were then investigated, in relation to whether the animal survived the bout of mastitis, using unconditional multivariate logistic regression. The final analyses involved comparison of the farmer questionnaire returns between the mastitic animal and the other cow from the same farm (exact conditional multivariate logistic regression was used). This matched case-control study was aimed at identifying risk factors associated with the occurrence of toxic mastitis.

The analyses investigated associations between the outcome of cases and cow risk factors to the occurrence of toxic mastitis. The information relating to cases where *Escherichia coli* was the only bacterial isolate was analysed as a separate subset of the data (as compared to the analysis where all cases were considered).

More details of the study design and analyses can be found elsewhere (14, 15, 16).
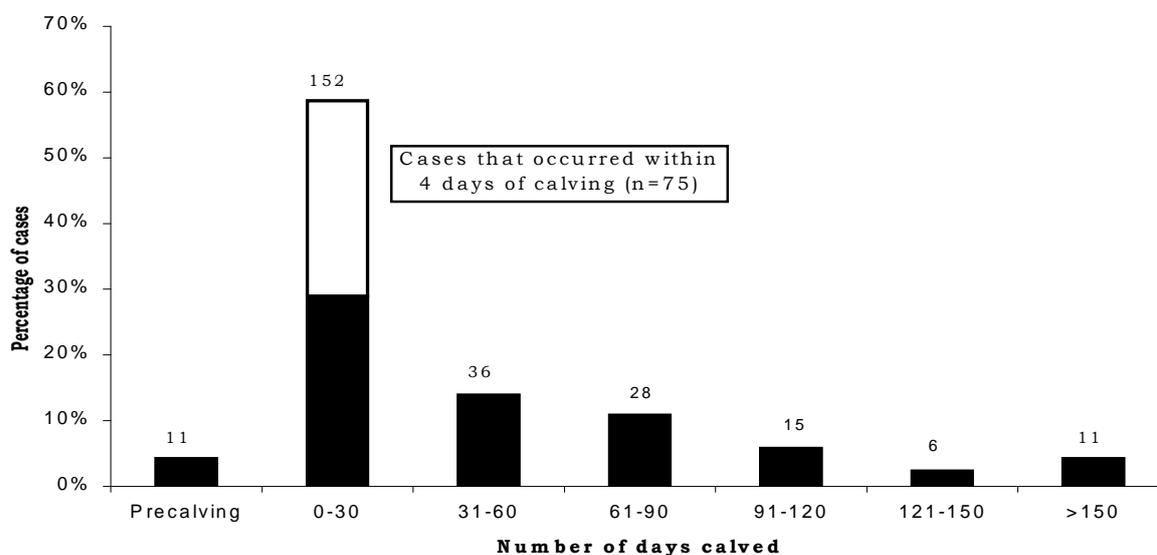
RESULTS

Information on 264 clinical cases was received from eighteen of the twenty veterinary practices asked to participate in the study between October 1995 and May 1997.

Occurrence

Almost 60% of cases occurred within the first month of calving with 29% of all cases occurring within four days of calving (Figure 1). Eleven cows (4%) developed clinical mastitis before they calved. March was the month of highest incidence (30% of all cases) with the vast majority of cases occurred during the winter housing period (84% of cases were reported during November to March). Interestingly, 14% of cases involved suckler cows.

Figure 1   Frequency distribution of cases of toxic mastitis in relation to the time of calving
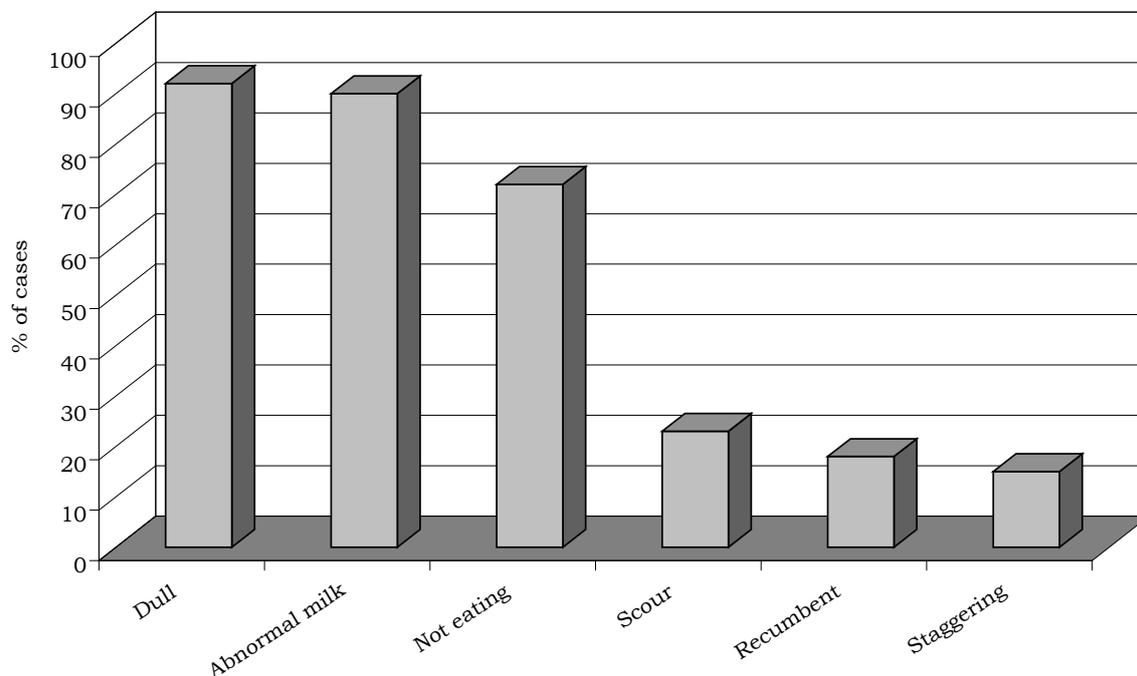

Clinical history

As illustrated in Figure 2, the main clinical signs reported from cases were dullness (92% of cases), abnormal milk (90%) and inappetance (72%). A further 23% of cases showed signs of diarrhoea while 18% were unable to stand.

Of the 224 cases where a calving history was reported, 72% calved without any assistance and only 4% of cases required veterinary attention for dystocia. Stillbirths were only reported in 2% of cases while 10% of cases retained the placenta for more than 24 hours. Concurrent disease was reported in 11% of cases with milk fever (43%) being the most common concomitant disease. No clinical disease in the month prior to calving was recorded in the vast majority of cases (91%). Veterinary attention was received within 12 hours of the animal being observed as ill for 70% of cases (17% received veterinary attention within 2 hours).

Figure 2   Percentage of toxic mastitis cases showing various clinical signs

Clinical and laboratory findings

Severe pyrexia (>40.6°C) was observed in 18% of cases with the vast majority (72%) showing either normal or slightly elevated rectal temperatures (38.3 to 40.6°C). Almost half of the cases (44%) showed signs of clinical dehydration as measured by the persistence of a fold of skin on the neck.

There was a significantly higher incidence of cases (p<0.001) where a hindquarter was affected with the majority of cases (88%) only having one quarter affected by the mastitis. Only 21% of farms with cases of acute toxic mastitis were considered to have a substandard level of hygiene on the premises.

Table 1   Milk sample characteristics obtained from cases of toxic mastitis

Characteristic		All samples (%)	Samples with Coliform isolate (%)
Colour	White, normal	41 (16)	26 (16)
	Yellow	171 (68)	119 (72)
	Other	40 (16)	20 (12)
Consistency	Normal	73 (29)	49 (30)
	Watery	120 (48)	81 (49)
	Serous or Viscous	59 (23)	35 (21)
Solids	None detected	23 (9)	19 (11)
	Floccules	71 (28)	46 (28)
	Flakes	74 (29)	52 (32)
	Clots	84 (33)	48 (29)

Table 2   Frequency of isolation of different bacteria from milk samples taken from cases of toxic mastitis.

Group	Bacterial isolate(s)	Number (%) of cases
Gram negative	*Escherichia coli*	132 (50.4)
	Citrobacter species	2 (0.8)
	Salmonella dublin	1 (0.4)
	Morganella morganii	1 (0.4)
	Proteus species	1 (0.4)
	Aeromonas species	1 (0.4)
	Pseudomonas species	3 (1.1)
	Mannheimia haemolytica	2 (0.8)
Gram positive	*Staphylococcus aureus*	7 (2.7)
	Streptococcus dysgalactiae	7 (2.7)
	Actinomyces species	7 (2.7)
	Streptococcus uberis	5 (1.9)
	Enterococcus species	2 (0.8)
	Staphylococcus xylosus	2 (0.8)
Fungus	*Candida* species	2 (0.8)
	Aspergillus species	1 (0.4)
Two isolates	*E. coli* & *Streptococcus uberis*	10 (3.8)
	E. coli & *Enterococcus* species	7 (2.7)
	E. coli & *Streptococcus dysgalactiae*	6 (2.3)
	Miscellaneous isolates	18 (6.9)
No bacteria isolated	-	30 (11.5)
More than 2 isolates	Contaminated	17 (6.5)

The milk samples were most frequently yellow, watery and contained clots (Table 1) but there was no statistical significance between the physical characteristics of the samples and the presence of coliform bacteria. Bacteriological examination of the milk samples indicated that half of the cases were due solely to *E. coli* infections (Table 2). A further fifteen organisms were isolated in pure culture contributing to 17% of all cases. Forty-one (16%) yielded growths of two bacterial species while 11% showed no bacterial growth and a further 6% were considered to be contaminated. Bacterial isolates from the milk samples commonly demonstrated antimicrobial susceptibility to enrofloxacin, framycetin and amoxycillin/clavulinic acid (16).

Examination of the blood samples submitted indicated abnormally high levels of plasma urea (31%) and plasma creatinine (42%) in a relatively large percentage of cases. Over half of the cases (56%) also showed very low levels of white blood cells (leucopenia) while only 18% showed an abnormally high packed cell volume (an indication of dehydration).
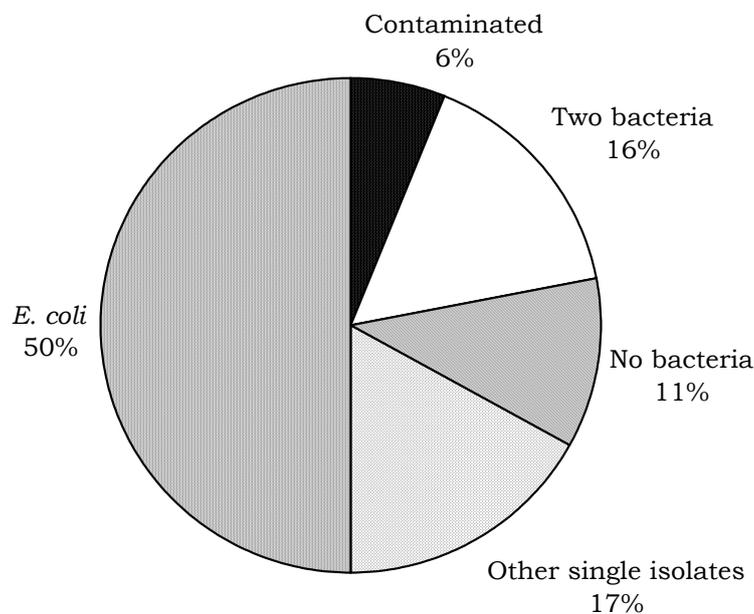
Therapy

Treatment was instigated by the farmer in 44% of cases prior to the visit from the veterinary surgeon (80% received intramammary antibiotic therapy and 60% were given parenteral injections). The vast majority of cases (97%) were given parenteral antibiotics by the attending veterinary surgeon and most (86%) also received anti-inflammatory drugs. Slightly over half of the cases (55%) received intramammary antibiotic therapy while one-third received oral fluids (32%). Only one-fifth of cases (22%) received intravenous fluids. Thirty-five per-cent of cases received other treatments, which were broadly equally split between vitamin B complex injections, parenteral calcium and oxytoxin.

Framycetin (38%) and amoxycillin/clavulinic acid (27%) were the most common parenteral antibiotics used by veterinary surgeons while flunixin meglumine (41%) was the main anti-inflammatory drug utilised. The median frequency of stripping of milk from the affected quarter was six times a day. Veterinary revisits to cases within 24 hours were planned in 48% of cases.
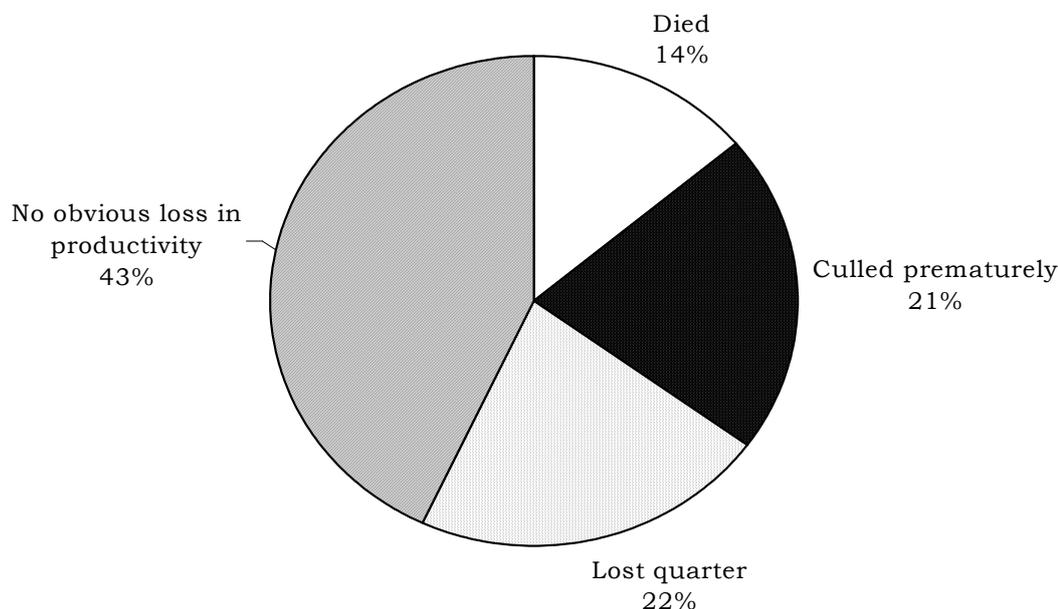
Case outcome

The outcome of the case was known in 219 instances in which 14% of cases died while a further 21% were culled early. Farmers also reported that 22% of cases lost the milk production from the affected quarter but the most common outcome (43%) was for the case to make an apparently uneventful recovery (Figure 3).

Figure 3   Summary of the bacterial isolates recovered from milk samples obtained from bovine toxic mastitis cases

The comparison of outcomes (died versus any of the other outcomes) for the 219 cases showed that cows which were recumbent at time of the first veterinary examination were eleven times more likely to die than those cows that could still stand. Furthermore, increases in the plasma creatinine levels at this time point were also associated with an increased risk of fatality. Figure 4 illustrates the risk of dying from toxic mastitis based on recumbency and the plasma creatinine level at the time of the first veterinary examination. For example, a recumbent cow suffering from toxic mastitis with a plasma creatinine level of 200 µmol/l has a 50% chance of dying from the condition.

Figure 4   Proportion of peracute and acute bovine toxic mastitis cases attributed to four different outcome classifications

Cow risk factors

From the information received from farmers, no management or disease risk factors were found to be associated with the occurrence of toxic mastitis with the 71 valid cases and matched controls. However, in a subset of these data (n=41) in which pure growths of *E. coli* were cultured from milk samples, administration of parenteral calcium at calving (p<0.01) and assistance at calving (p<0.01) were both associated with toxic mastitis. The risk of a cow developing *E. coli* mastitis was 23 times higher if it had received parenteral calcium. If the cow had received assistance at calving, then the risk of dying was increased eleven fold.

DISCUSSION

These 264 clinical cases of acute toxic mastitis, which were reasonably well-distributed across Northern Ireland, were considered to originate from a satisfactory representation of the Province's cow population and therefore the results could be extrapolated to the country as a whole.

The seasonal distribution of cases is similar to that observed elsewhere (1, 9) and the relationship between occurrence of toxic mastitis and the peri-

parturient status has been described by others (9, 19). This highlights the need for extra vigilance during the period immediately after calving, particularly as toxic mastitis can rapidly become life threatening, if is not detected and treated promptly during this period. March was the month of highest incidence (30% of all cases). This probably reflects the build up over the winter of environmental sources of the bacteria, which are responsible for the condition. The vast majority of cases occurred during the winter housing period (84% of cases were reported during November to March). Maintaining clean, dry cattle houses is essential in helping to reduce udder contamination and hence the occurrence of toxic mastitis.

It was noteworthy that 14% of cases involved suckler cows. This may be partly due to the significant proportion of Friesian/Holstein cross-breds utilised as suckler cows. Additionally, there has been further intensification of suckler herd management over recent years, particularly in relation to housing.

The relatively large sample size has also enabled estimation of the relative frequency of various presenting signs and clinical findings to be evaluated. Similarly, the frequency of use of various therapies to treat cases has been assessed and the findings are comparable with those recorded elsewhere (A.H. Andrews, unpublished data).

The relative frequency of clinical signs commonly shown by cows with toxic mastitis were lethargy (92%), abnormal milk (90%) and anorexia (72%). The fact that hind-quarters were more commonly affected are consistent with the findings elsewhere (18, 20) and the fact that hind-quarters are closer to the sources of environmental bacteria by virtue of being next to the tail and hind-legs as well as closer to the ground than the fore-quarters, makes this a biological plausible finding.

At first appearance, the subjective assessment that cases occurred more frequently on farms with a high standard of hygiene seems contrary to the belief that reduction in the level of environmental contamination of the udder will reduce the incidence of toxic mastitis. However, other studies (3, 8) have shown relationships between the incidence of toxic mastitis and low bulk milk cell counts and the standard of hygiene may simply be a confounding observation. Moreover, aesthetic cleanliness is not necessarily a good indicator of the bacteriological state of the environment which is more dependent upon moisture content.
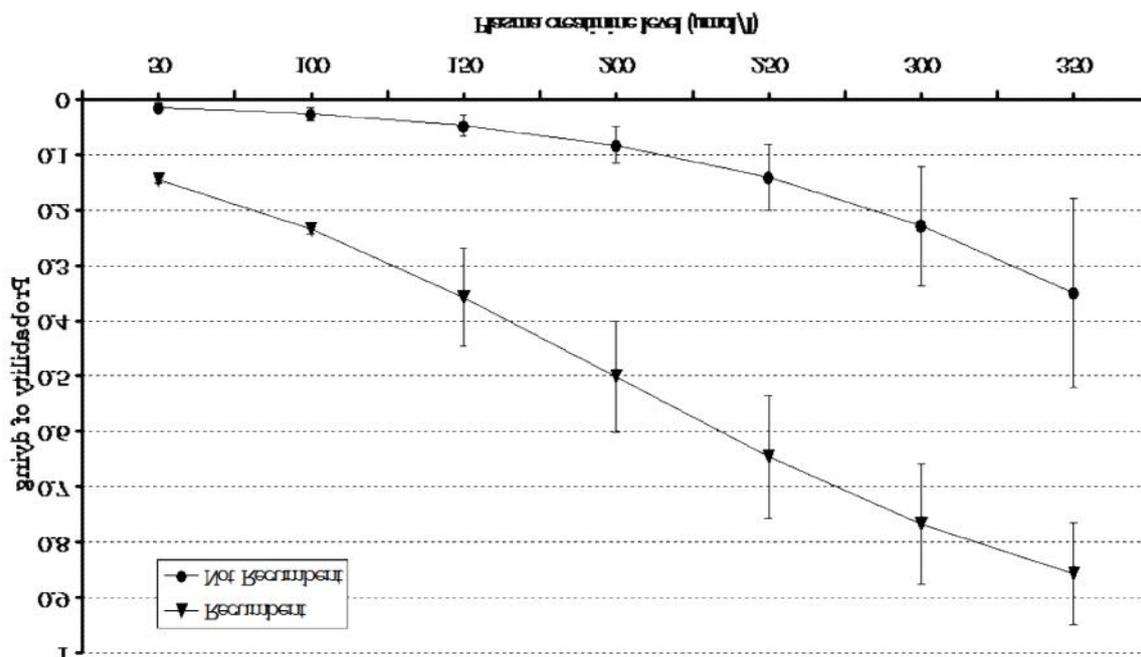
Recovery of pure growths of *E. coli* from half of the milk samples along with work reported from other studies confirms the belief that toxic mastitis is mainly a condition caused by coliform bacteria (2, 7). Many of the bacterial isolates tended to be sensitive to the antibacterial agents used to treat such cases although the relative sensitivities have changed over the last 20 years (12, 20). Milk samples commonly were reported as having a yellow, watery appearance with clots but there was no association between the appearance

and the bacteria isolated. However, other workers have found differences in the appearance of milk and the bacteria involved (17).

The laboratory findings of high levels of plasma urea (31%) and creatinine (42%) along with low levels of white blood cells (leucopenia 56%) have been reported elsewhere (5, 11, 21).

The case outcomes (14% case fatality rate and 21% culled prematurely) were similar to those reported by Jones and Ward (10). However, another study (6) reported only 24% of cases either dying or being culled prematurely. The 50% fatality rate in cows that were recumbent was comparable with a recent regional study (5) in which 46% of recumbent cows died. Furthermore, the use of the information gathered has enabled a prognostic indicator model to be developed (Figure 5). This model will be of practical assistance to the veterinary surgeon and farmer in deciding on the best course of action to take with toxic mastitis cases since recumbent animals have a much more guarded prognosis. Interestingly, none of the various forms of treatment appeared to affect the outcome of the cases significantly associated. This is probably due to the reasonably standard treatment approach utilised across the different veterinary practices rather than a reflection on any ineffectual nature of any of the therapies. Another recent study (4) also found no significant difference between treatment regimens.

Figure 5   Probability of dying from toxic mastitis based on ability to stand and plasma creatinine level

Analysis of information supplied by farmers indicated that where a cow required assistance at calving or was thought to have milk fever, the risk of such cows developing toxic mastitis was increased. Therefore, measures that would decrease the number of difficult calvings and reduce the occurrence of milk fever, would also help to reduce the occurrence of toxic mastitis within a herd.

CONCLUSIONS

The results of this study present a comprehensive description of the clinical and laboratory findings from clinical cases of toxic mastitis amongst a random selection of cows. In general, the findings were consistent with those reported from other field studies but this study did provide an improved quantitative assessment of the different parameters that were measured. Furthermore, attention to detail and good husbandry practices to prevent calving problems and milk fever should help to reduce the incidence of toxic mastitis within a herd. Finally, the study has also provided information which will accurately predict the outcome of cases which is important in assisting in the decision making process for such cases as aggressive therapy in the treatment of this condition can be expensive.

ACKNOWLEDGEMENTS

REFERENCES

1.   Andrews, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G. (1992) Bovine medicine: diseases and husbandry of cattle. Blackwell Scientific Publications, Oxford pp 922.
2.   Bradley, A.J. and Green, M. (2001) Aetiology of clinical mastitis in six Somerset dairy herds. *Vet. Rec.* **148**: 683-686.
3.   Green, M.J., Green, L.E. and Cripps, P.J. (1996) Low bulk milk somatic cell counts and endotoxin-associated (toxic) mastitis. *Vet. Rec.* **138**: 305-306.
4.   Green, M.J., Green, L.E. and Cripps, P.J. (1997) Comparison of fluid and flunixin meglumine therapy in combination and individually in the treatment of toxic mastitis. *Vet. Rec.* **140**: 149-152.

5.   Green, M.J., Cripps, P.J. and Green, L.E. (1998) Prognostic indicators for toxic mastitis in dairy cows. *Vet. Rec.* **143**: 127-130.

6.   Golodetz, C.L. and White, M.E. (1983) Prognosis for cows with severe clinical coliform mastitis. *Vet. Rec.* **112**: 402-403.

7.   Hazlett, M.J., Little, P.B., Maxie, M.G. and Barnum, D.A. (1984) Fatal mastitis in dairy cows: A retrospective study. *Can. J. Comp. Med.* **48**: 125-129.

8.   Lam, T.J.G.M., Schukken, Y.H., van Vliet, J.H., Grommers, F.J., Tielen, M.J.M. and Brand, A. (1997) Effect of natural infection with minor pathogens on susceptibility to natural infection with major pathogens in the bovine mammary gland. *AJVR* **58**: 17-22.

9.   Jones, T.O. (1990) *Escherichia coli* mastitis in dairy cattle – A review of the literature. *Vet. Bull.* **60**: 206-231.

10.   Jones, G.F. and Ward, G.E. (1990) Evaluation of systemic administration of gentamicin for treatment of coliform mastitis in cows. *JAVMA* **197**: 731-735.

11.   Katholm, J. and Haubro Anderson, P.H. (1992) Acute coliform mastitis in dairy cows: endotoxin and biochemical changes in plasma and colony-forming units in milk. *Vet. Rec.* **131**: 513-514.

12.   Mackie, D.P., Logan, E.F., Pollock, D.A. and Rodgers, S.P. (1988) Antibiotic sensitivity of bovine staphylococcal and coliform mastitis isolates over four years. *Vet. Rec.* **123**: 515-517.

13.   Menzies, F.D., Bryson, D.G., McCallion, T. and Matthews, D.A. (1995) A study of mortality among suckler and dairy cows in Northern Ireland in 1992. *Vet. Rec.* **137**: 531-536.

14.   Menzies, F.D., Gordon, A.W., McBride, S.H. and Goodall, E.A. (2003) Risk factor for toxic mastitis in cows. *Vet. Rec.* **152**: 319-322.

15.   Menzies, F.D., Gordon, A.W., McBride, S.H. and Goodall, E.A. (2003) Prognostic indicators for bovine toxic mastitis: results from a study in Northern Ireland. *Vet. Rec.* (submitted).

16.   Menzies, F.D., McBride, S.H., McDowell, S.W.J., McCoy, M.A., McConnell, W. and Bell, C. (2000) Clinical and laboratory findings in cases of toxic mastitis in cows in Northern Ireland. *Vet. Rec.* **147**: 123-128.

17.   Milne, M.H., Biggs, A.M., Fitzpatrick, J.L., Innocent, G.T. and Barrett, D.C. (2003) Use of clinical information to predict the characteristics of bacteria isolated form clinical cases of bovine mastitis. *Vet. Rec.* **152**: 615-617.

18.   Miltenburg, J.D., de Lange, D., Crauwels, A.P.P., Bongers, J.H., Tielen, M.J.M., Schukken, Y.H. and Elbers, A.R.W. (1996) Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands. *Vet. Rec.* **139**: 204-207.

19.   Oz, H.H., Farnsworth, R.J. and Larson, V.L. (1985) Environmental mastitis. *Vet. Bull.* **55**: 829-840.

20.   Pearson, J.K.L. and Mackie, D.P. (1979) Factors associated with the occurrence, cause and outcome of clinical mastitis in dairy cattle. *Vet. Rec.* **105**: 456-463.

21.   Radostits, O.M., Blood, D.C. and Gay, C.C. (1994) Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. Baillière Tindall, London pp 1763.

Exploiting the genome in the control of *Streptococcus uberis*

James Leigh

Institute for Animal Health Compton Laboratory, Compton, Newbury, Berks RG20 7NN
Email: james.leigh@bbsrc.ac.uk

SUMMARY

Streptococcus uberis is a common cause of bovine mastitis world-wide. Approaches that have controlled infection with other pathogens have had little impact on the incidence of disease due to infection with this bacterium and vaccines have been suggested as means of control. Information on which antigens of the bacterium should be included in vaccines is not available and further research is required to identify such candidates.

The genome sequence of *S. uberis* is nearing completion. This data, alongside various recent technical developments that have enabled the production of mutant strains in which specific genes can be inactivated and/or altered, will permit the identification of bacterial components that are *essential* for infection of the bovine mammary gland. Production of such data, coupled to knowledge of the disease pathogenesis, will result in the identification of factors that can be used in effective, sub-unit vaccines in the control of *S. uberis* infection.

INTRODUCTION TO THE SPECIES

The identification of streptococci as a cause of bovine mastitis was first recorded towards the end of the 19th century. However, it was not until the 1920s that *S. uberis* was described as a discrete pathogen of the bovine mammary gland. The bacterium only received its status as a separate species following the work of Diernhofer in 1932, who was also the first to coin the species name, *uberis*. In the late 1970s, the original species was shown to contain two discrete genotypes (types I and II). The latter was allocated a separate species status (*Streptococcus. parauberis*) in 1990 and has subsequently been shown to be a cause of mastitis only rarely (2). Detailed analysis, conducted in the early 1990s, placed *S. uberis* amongst the largest intra-generic grouping of *Streptococcus* that is centred on the human pathogen, *Streptococcus pyogenes*, but also contains a number of other streptococci that are associated with disease in a variety of animal species (3). Consequently, *S. uberis* can be considered to share an evolutionary history with a number of other pathogenic streptococci, capable of causing various diseases in a number of animal species, including man.

S. uberis is readily isolated from many body sites of the dairy cow, but is rarely associated with diseases outside the mammary gland. In addition to causing mastitis in the dairy cow, *S. uberis* has also been implemented as a

cause of mastitis in the sheep, goat and buffalo. *S. uberis* has only very rarely been implicated as a cause of disease in other species.

S. uberis has been isolated from many sites of the cow including the tonsils, genital tract, rumen, rectum and coat and can be isolated in high numbers from bedding used by cattle. The particular reservoir of the bacterium that acts as the source of infectious agent from the mammary gland is uncertain. Indeed, it is unlikely that any one site of the cow is the key reservoir in all cases. Cows at pasture are often considered to be at lower risk of infection by *S. uberis* than housed cattle. However, *S. uberis* has been isolated from heavily used pasture in numbers similar to those seen in bedding materials. In addition, in New Zealand where animals are housed for only very short periods of time, if at all, *S. uberis* is one of the most significant causes of bovine mastitis and occurs at a rate similar to that found in the UK. *S. uberis* is found as a pathogen of the bovine mammary gland in many geographical regions, however, the incidence of infection may vary widely from region to region and even between herds in similar locations. In many areas of the world the implementation of the five-point plan for the control of bovine mastitis has had a positive impact in reducing the incidence of clinical mastitis, however, in no instance has this eliminated intramammary infection by *S. uberis*. It is widely accepted that in addition to the possibility of cow to cow, or quarter to quarter transmission during milking, a significant number of infections due to *S. uberis* arise from a reservoir of the bacterium present in the environment of the dairy cow (for review see (6))

It would appear, therefore, that in herds where contagious routes of transmission are being controlled adequately, infection of the bovine mammary gland by *S. uberis* is likely to result from bacterial sources in the local environment.

New products and/or procedures are required to reduce the incidence of mastitis caused by this bacterium and vaccines have been highlighted as a means of disease control. The production of such products will benefit from greater knowledge of the precise interactions between *S. uberis* and the bovine mammary gland that permit colonisation and disease. The era of genome based research will assist the speed with which such knowledge may be acquired and will provide insights into interactions that would otherwise be overlooked. By targeting our approach it will be possible to exploit this new era of biological science to identify key processes in the interaction between *S. uberis* and the mammary gland and identify key targets against which effective vaccines can be made.

THE *Streptococcus uberis* GENOME PROJECT

S. uberis like most other bacteria has a single circular chromosome, unlike a number of other bacteria it has not been shown to contain any extra-chromosomal DNA. Sequencing of the genome of *S. uberis* commenced in November 2001 at the Sanger Institute in the UK. The organism chosen for

sequencing was strain 0140J. This was isolated by staff at the National Institute for Research in Dairying (NIRD) from a case of clinical bovine mastitis from a lactating cow in the UK in 1972. This organism has been the subject of much intensive investigation at IAH and it is also known to be infectious for both the lactating and non-lactating mammary gland (5)

Sequencing of bacterial genomes occurs in several stages. The first phase is shotgun cloning and sequencing. Chromosomal DNA isolated from *S. uberis* 0140J was broken into fragments, inserted into a vector (piece of circular DNA (plasmid) that replicates to produce a high number of copies within a bacterial host cell) and amplified in laboratory strains of *Escherichia coli*. Each vector, containing a different fragment of *S. uberis* DNA, was isolated and the fragment sequenced. To ensure that full coverage of the genome was achieved this was repeated for over 26,000 different fragments. The DNA sequences obtained (shotgun sequences) then entered the Assembly phase. All the sequences were compared to find regions where they overlap. The overlapping sequences were assembled to form contiguous sequences or "contigs", which were then re-analysed to determine regions that overlap with other contigs and other shotgun sequences. Several rounds of the assembly phase were carried out. This was followed by a Finishing or Gap-Closure phase. This stage of the process is currently underway. Finishing is achieved by determining the DNA sequence that lies between each adjacent contig. However, the order of the contigs with respect to the chromosomal DNA sequence is not known. Consequently, the reaction has to be conducted independently from each contig to every other contig. This is accomplished in a high-throughput manner by a process known as combinatorial polymerase chain reaction (PCR). (To review progress see: http://www.sanger.ac.uk/Projects/S_uberis/).

Currently (July 2003), 1.8 Million bases of DNA sequence are assembled into 63 contigs and finishing is well underway. Once completed, each gene within the circular chromosome will be compared to _every_ known gene and where possible, putative function will be ascribed based on its similarity to genes of known function.

Despite not being completed, the existing sequence data is already a valuable resource for researchers and has already facilitated the investigation into the interaction between *S. uberis* and the bovine host.

CURRENT USES OF THE *Streptococcus uberis* GENOME

For many years the function of genes and proteins in bacteria has been studied by exploiting the differences between strains that differ in key properties. Both field isolates and defined mutant strains have been used for this purpose. The use of field strains can, however, lead to an inaccurate interpretation of results based on the limited knowledge available at the time of investigation. For instance, in the case of *S. uberis*, the capsule surrounding the bacterial cell was considered to be important during

infection. This followed three key observations that showed that capsule was usually present on isolates from clinical disease (1), encapsulated strains were less likely to be killed by neutrophils (during laboratory tests) (1, 7) and a non-capsular field strain (EF20) was less able to infect the mammary gland and cause mastitis than a capsulated field strain (0140J) (5). However, subsequent research revealed that a non-capsular mutant strain of 0140J (TRF006) was in fact fully virulent, leading to the more accurate conclusion that the capsule plays little, if any, role during the pathogenesis of mastitis within the lactating mammary gland (4). Recent data indicates that *S. uberis* avoids being taken up and killed in the mammary gland by directly inhibiting neutrophils through the release an unknown/unidentified factor.

The genome may be exploited in any number of ways, but in the case of assessing the role of particular gene and its encoded protein, mutational analysis coupled to an assessment phase is the most common approach. Following a number of technical and scientific developments (8), it is now possible to genetically manipulate *S. uberis*. Genes in this bacterium can now be inactivated, generating mutant strains that fail to express the respective encoded protein (9, 10, 12). This can be achieved either by random or targeted mutagenesis. The capability of the resulting mutant strains to cause disease can then be compared to that of the intact parent strain in a number of model systems *in vitro* and ultimately by evaluation of virulence through the use of experimental challenge models in the dairy cow.

How do we know which genes we have inactivated?

It has been possible to screen banks of mutant strains to identify those that have lost a particular activity/property (phenotype). In three published examples of this approach we have isolated groups of mutant strains with altered phenotypes, these mutants either lacked capsule (12), failed ultilise peptides as a source of amino acids (9) or failed to grow in milk due the inability to obtain trace elements (10). In each case, comparing a short stretch of the DNA sequence flanking the site of mutation and comparing this to the genome sequence allowed the rapid identification of both the mutated gene and its known function in other bacteria. Such an approach has identified the genes responsible for the production and regulation of capsule, the uptake of trace elements from milk and the uptake of peptides. Subsequent evaluation *in vivo* showed that capsule was irrelevant to disease but that growth in milk was essential.

How do we know which genes may be useful in vaccines?

Isolation of further mutants with altered phenotypes relevant to disease will identify more genes required for the existence of *S. uberis* within the mammary gland of the dairy cow. The precise role of each gene could be evaluated however such an approach would be both a time consuming and expensive way in which to identify potential vaccine antigens. Furthermore,

such candidates would require a great deal of analysis to determine their potential experimentally.

Sufficient knowledge of protein function exists to predict from their sequence alone those that may be present at the surface or secreted by the bacterial cell. These proteins are more likely to interact with the bovine host and, more importantly, will be accessible to host's immune response than those present within the cell. So, genomic sequence data can be used to not only to identify the inactivated genes in mutants lacking functions relevant to disease, but can also be used to determine whether the mutated gene produces a protein that is likely to be accessible to the host immune response. In addition, as the isolates of *S. uberis* that cause disease are a diverse group of bacteria, it is possible to determine firstly, whether this gene is present in other disease isolates and to determine its variation within the population.

Of course, these activities could be performed in the absence of a specific genome sequence for *S. uberis*, however availability of the genome has reduced the time required for this process by several orders of magnitude. Whereas in the past, scientists could spend an entire career looking at the function of only a few proteins from a bacterium, it is now possible to look at an entire "genome's-worth" of proteins within the time frame of a scientific grant, typically 3-5 years.

Screening procedures based on phenotypic changes make several assumptions. Firstly, that the screening procedure matches some attribute that is relevant *in vivo*. Secondly, that a screening protocol can be developed and finally that a single gene is responsible for the production of a particular phenotype. This is not always the case. For instance, the screen for capsule in *S. uberis* although simple to develop has proved to be irrelevant to the situation *in vivo* where it is unlikely that capsule is produced. The need to develop a screening protocol to isolate mutants of *S. uberis* unable to inhibit neutrophil function has proved very difficult and has not yet been established. However, the protocol based on growth in milk has proved useful in the isolation of avirulent mutants and has enabled the identification of potential vaccine targets.

Can the genome be used to direct the search for vaccine targets?

The simple answer is yes, but there are many ways in which this can be achieved.

At its most simple level, the genome can be screened to detect all genes of known function that fulfil criteria for vaccine antigens. For instance surface proteins can be identified by virtue of the presence of specific sequences. In Gram-positive bacteria, such as streptococci, proteins may be anchored at the cell surface by a number of mechanisms, one of which is through the action of an enzyme called sortase; so named as it "sorts" other proteins to the cell surface. All the proteins that act as substrates for sortase carry the

amino acid sequence LPXTG or LPXXG (where X is any amino acid) towards one end of the sequence (11). The existing genome sequence has been screened and 36 genes encoding such sequences have been uncovered in *S. uberis*. These have a range of functions and some are of unknown function. It would of course be possible to isolate mutants in which each of these genes were inactivated individually, *however*, we can study the collective role of all these protein by analysis of a single mutant in which the gene encoding sortase has been inactivated. This investigation is currently underway at IAH.

Similarly, the role of groups of proteins that are processed by other pathways that permit secretion or expression at the surface can be investigated by exploiting mutants in which any of the genes responsible for the pathway itself have been inactivated.

Bacteria tend to regulate expression from genes so that only those appropriate for any particular environment are expressed. This can be exploited experimentally by growing bacteria in different conditions and determining those genes that are switched on in one condition and compared to the other. The output data from such an analysis can be produced in a number of ways, that do not require review here, but in each case the output data can be related directly to the genome sequence and used to identify genes that are switched on or off. This is particularly relevant for genes that may only be expressed during infection and which, therefore may be surmised to play a role in pathogenesis.

In a similar situation, many genes, which are known to act as regulators of other genes have been identified in bacteria. Regulatory genes have also been located in the *S. uberis* genome and a number of mutants in which these are inactivated have been isolated. Screening of these mutants for changes that may be associated with loss of virulence and indeed determining the virulence of such mutants to the wild type strains will permit the identification of regulators which control expression of virulence genes. In a manner similar to that described above comparisons of genes that are expressed differently in such strains would then allow the identification of subsets of genes and their products that are required for infection. This approach to the identification of potential vaccine targets is also underway at IAH.

Following completion of the current genome from a known virulent strain of *S. uberis* (0140J) it will be possible to sequence further less virulent or avirulent strains, such as EF20, and make a direct comparison of the genetic differences between these two organisms. Genes present in 0140J and absent or altered in EF20 hold the genetic coding for virulence. Again, analysis of these differences with a view to the identification of factors that are accessible to the immune system will provide greater insight into potential vaccine candidates.

CONCLUSION

It is now true to say that research into the discovery of vaccine candidates for *S. uberis* has reached the era of modern molecular sciences. Although not exhaustive, the preceding list of approaches that can now be employed in this search shows how far research in this area has developed in a short space of time. Only two years ago very little of this was possible.

Exploitation of these new tools, alongside existing knowledge of disease and experimental models in which the disease, or aspects of the disease, can be replicated has already enabled existing hypotheses to be tested and disproved/confirmed. Careful focussing of the approach taken by researchers in the future will generate data that will lead to the identification of new and novel candidates that may be used in effective vaccines against *S. uberis*. The speed with which this can be achieved, now more than ever before, will depend on the level of co-ordinated investment and the discipline to conduct thorough science-led investigations towards this single important aim.

ACKNOWLEDGEMENTS

REFERENCES

1.   Almeida, R.A. and Oliver, S.P. (1993) Antiphagocytic effect of the capsule of *Streptococcus uberis*. *J. Vet. Med.* **40**: 707-714.
2.   Bentley, R.W., Leigh, J.A. and Collins, M.D. (1993) The development and use of species specific oligonucleotide probes for the differentiation of *Streptococcus uberis* and *Streptococcus parauberis*. *J. Clin. Microbiol.* **31**: 57-60.
3.   Bentley, R.W., Leigh, J.A. and Collins, M.D (1991) Intrageneric structure of *Streptococcus* based on comparative analysis of small sub-unit rRNA sequences. *Int. J. Syst. Bacteriol.* **41**: 487-494.
4.   Field, T.R., Ward, P.N., Pedersen, L.H. and Leigh, J.A. (2003) The hyaluronic acid capsule of *Streptococcus uberis* is not required for the development of infection and clinical mastitis *Infect. Immun.* **71**: 132-139.
5.   Hill, A.W. (1988) Pathogenicity of two strains of *Streptococcus uberis* infused into the lactating and non-lactating mammary gland. *Res.Vet. Sci.* **45**: 400-404.

6.   Leigh, J.A. (1999) *Streptococcus uberis*: A permanent barrier to the control of bovine mastitis? (A Review) *Vet. J.* **157**: 225-238.

7.   Leigh, J.A. and Field, T.R. (1994) *Streptococcus uberis* resists phagocytosis despite the presence of bound immunoglobulin. *Infect. Immun.* **62**:1854-1859.

8.   Maguin, E., Prevost, H., Ehrlich, S.D. and Gruss, A. (1996) Efficient insertional mutagenesis in lactococci and other gram-positive bacteria. *J. Bacteriol.* **178**: 931-935.

9.   Smith, A.J., Kitt, A.J., Ward, P.N. and Leigh, J.A. (2002) Isolation and characterization of a mutant strain of Streptococcus uberis, which fails to utilize a plasmin derived beta-casein peptide for the acquisition of methionine. *J. Appl. Microbiol.* **93**: 631-9.

10.   Smith, A.J., Ward, P.N., Field, T.R., Jones, C.L., Lincoln, R.A. and Leigh, J.A. (2003) MtuA, a lipoprotein receptor antigen from *Streptococcus uberis*, is responsible for acquisition of manganese during growth in milk and is essential for infection of the lactating bovine mammary gland *Infect. Immun.* **71**: in press

11.   Ton-That, H., Liu, G., Mazmanian, S.K., Faull, K.F., Schneewind, O. (1999) Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of Staphylococcus aureus at the LPXTG motif. *Pro. Nat. Acad. Sci. USA* **26**:12424-12429.

12.   Ward, P.N., Field, T.R., Ditcham, W.G., Maguin, E. and Leigh, J.A. (2001) Identification and disruption of two discrete loci encoding hyaluronic acid capsule biosynthesis genes hasA, hasB, and hasC in *Streptococcus uberis. Infect. Immun.* **69**: 392-399.

CORYNEBACTERIUM BOVIS – FRIEND OR FOE?

Jon N. Huxley[1], Martin J. Green[2] and Andrew J. Bradley[1]

[1] Division of Farm Animal Science, University of Bristol, Langford House, Langford, Bristol BS40 5DU
Email: Jon.Huxley@bristol.ac.uk

[2] Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL/Orchard Veterinary Group, Wirral Park Road, Glastonbury, Somerset BA6 9XE

SUMMARY

Corynebacterium bovis is one of the most prevalent organisms isolated from bovine milk. However, its presence is often ignored because of its limited pathogenicity. This paper reviews the available literature and describes the characteristics, location of infection, pathogenicity, prevalence and control of *C. bovis.* It has been postulated that infection with *C. bovis* may protect quarters against subsequent infection with other more pathogenic organism, the available literature on this subject is discussed and the potential methods by which *C. bovis* may afford protection are outlined.

Work on *C. bovis* conducted at the University of Bristol is described. A novel method of identification based on endonuclease restriction analysis of the 16S rRNA gene sequence is outlined. A data set collected during a prospective dry cow study was analysed to assess the significance of *C. bovis* infection in and around the dry period. Infection with *C. bovis* was associated with a significant elevation in somatic cell count (SCC). However, quarters infected with *C. bovis* were significantly less likely to be concurrently infected with major pathogens and quarter which retained a *C. bovis* infection during the dry period were significantly less likely to acquire a major pathogen infection during that time. Preliminary work on the methods by which *C. bovis* protects quarters are discussed.

INTRODUCTION

Characteristics and location of infection

C. bovis is a member of the genus Corynebacterium. Corynebacterium species are aerobic, asporogenous, non-partially-acid fast, irregularly shaped, Gram-positive rods (15), which often demonstrate a characteristic "club" shape. *C. bovis* is almost exclusively isolated from bovine sources (15), primarily the mammary gland, although it has been reported in other locations (12). It belongs to a genus sub-grouping (composed of 10 species) whose principal characteristic is that their growth *in vitro* is enhanced by the addition of lipid to growth media. These species are referred to as "lipophilic" (15).

The primary site of *C. bovis* infection within the bovine mammary gland is thought to be the teat canal (5, 32). Numbers of organisms appear to fall rapidly above and around Furstenberg's rosette (5, 32), although infection can exist in the teat cistern and udder tissues (31).

Pathogenicity

C. bovis is considered a pathogen of limited significance, primarily causing sub-clinical disease (5, 6, 24). Some authors even consider it should be termed a commensal (7). Despite its limited pathogenicity it is highly infectious (6), even more so than *Staphylococcus aureus* and *Staphylococcus agalactiae* (the classic contagious pathogens) (32).

Analysis of large amounts of data from the available literature suggests that both natural and artificial infection with *C. bovis* elevates quarter SCC by approximately 50,000 cells/ml. More recently a study in the USA on 893 herds concluded that for every one- percent increase in cow level prevalence the BMSCC was elevated by 1,091 cells/ml (43).

C. bovis as a cause of clinical disease

C. bovis and *Corynebacterium* species have occasionally been reported as the cause of both individual cases and "herd outbreaks" of clinical mastitis (11, 13, 20, 30, 35). However, there does appear to be some uncertainty over the identification of the causal organism, especially in the case reported by Cobb and Walley (11).

In cases where *C. bovis* is described as a cause of clinical disease, most authors discuss the possibility that the presence of *C. bovis* in a mastitic sample may in fact be a "no growth" case of clinical mastitis in which the *C. bovis* is an incidental finding. In many herds a high proportion of milk samples from clinically normal animals are *C. bovis* positive. It can be expected that the same proportion of clinical mastitis samples in these herds will also be *C. bovis* positive and thus unrelated to the clinical disease.

Prevalence of *C. bovis* in dairy herds

Data on the prevalence of *C. bovis* in lactating cows from the UK and around the world and over the last 30 years prove remarkably consistent and suggest that approximately 20 to 30 percent of quarters are infected at any one time (9, 19, 23, 28, 34, 36). These figures make *C. bovis* along with the coagulase negative staphylococci (CNS, also considered minor pathogens) the most prevalent organisms isolated from the bovine mammary gland.

Control of *C. bovis*

Despite its high prevalence in many herds its limited pathogenicity has meant that few specific recommendations for the control of *C. bovis* have been formulated. It is, however, one of the few truly "contagious" pathogens

of the bovine mammary gland and, as such, measures designed to control *S. aureus* and *S. agalactiae* have also proved effective against *C. bovis*.

Dry cow therapy (DCT) is an efficient method of eliminating existing *C. bovis* IMI (intramammary infection). Cure rates of 96.3%, 96.0%, 92.6% and 73.8% (24), 100%, 100% and 94.1% (18) and 75% (39) have been reported with a wide range of dry cow antibiotic formulations.

Post-milking teat disinfection (PMTD) eliminates any contagious pathogens from the teat surface and distal teat orifice, which may have been transferred there from infected cows during the milking process. It effectively prevents much of the cow-to-cow transmission that would otherwise occur. Lam and colleagues (28) conducted a field study on seven farms in Holland, in which PMTD was discontinued on half the teats of all cows. The prevalence of *C. bovis* IMI was significantly lower at drying off (47.2% *cf.* 63.6%) and calving (26.2% *cf.* 36.81%) in dipped quarters. During lactation, the incidence of new (28.5 *cf.* 50.2 cases per 10,000 quarter days at risk) and duration of existing (186.7 *cf.* 236.8 days) *C. bovis* infections was significantly decreased in dipped quarters. Comparing Canadian herds that practiced PMTD and DCT to those that only practiced DCT, Brooks and colleagues (9), demonstrated a reduction in prevalence at both the quarter (11.5% *cf.* 35.9%) and cow (22.0% *cf.* 60.7%) levels. A high prevalence of *C. bovis* within a herd is often regarded as a marker of poor implementation of PMTD.

Although it is impossible to isolate the effect of each control measure, Bramley and colleagues reported on the effects of introducing routine DCT and PMTD onto 30 farms with a total of 2000 cows. Within three years the quarter prevalence of *C. bovis* had dropped from 47 to five percent (6).

Protective effect

The role of *C. bovis* in IMI dynamics is contentious. As long ago as 1972 it was suggested that *C. bovis* may play a role in protecting quarters against subsequent infection with other pathogens (4). Since that time the literature has produced much conflicting data on the subject.

Studies employing an experimental design based on artificial infection models have concluded that quarters infected with *C. bovis* are less likely to become infected with *S. aureus* (8, 29, 32, 38). However, no effect was demonstrated against *Streptococcus uberis* (14) and *S. agalactiae* (8). In another study the authors concluded quarters infected with *C. bovis* were actually at increased risk of becoming infected with *S. agalactiae* (32).

Studies employing an experimental design based on natural infections have concluded that quarters infected with *C. bovis* were less likely to become infected with *S. uberis* (27), *S. agalactiae* (34) and all major pathogens (4, 27, 33) and were partially protected against *S. aureus* (44). In other studies no protective affect was demonstrated against *S. aureus* (3, 41) *S. agalactiae*

(41), *S. uberis* (44), *Escherichia coli* (21) and major pathogens (24). Conversely other authors have concluded that quarters infected with *C. bovis* were at significantly greater risk of suffering concurrent infection with environmental Streptococci (3, 21) and *S. aureus, S. agalactiae,* other Streptococci and all major pathogens (9).

Recently, Green and colleagues have investigated the effect of infection with Corynebacterium species in and around the dry period on the incidence of clinical mastitis. Quarters infected with Corynebacterium species in the late dry period or the week following calving were at significantly less risk of suffering clinical mastitis during the whole lactation. Interestingly however, quarters infected with Corynebacterium species at drying off were at significantly greater risk of suffering clinical mastitis during the next lactation (17).

On balance the authors concludes that the literature is suggestive of a protective effect associated with *C. bovis* infection (especially against *S. aureus*) although the evidence is far from conclusive.

Mode of protective effect

If quarters infected with *C. bovis* are protected against subsequent infection with more pathogenic organisms, a number of possible explanations for this effect have been postulated, including competitive growth/antagonism, induced leucocytosis or an increase in host immunity (4).

Quarters with higher SCC were at significantly lower risk of become infected following artificial infection with *S. aureus* (38) and *Aerobacter aerogenes* (37). More recently it has been demonstrated that quarters with very low SCC (<20,000 cells/ml) are at increased risk of suffering clinical mastitis compared to quarters with higher SCC (20,000–60,000 cells/ml) (16). Interestingly the increase in SCC in quarters infected with *C. bovis* (approximately 50,000 cells/ml higher than those that are bacteriologically negative) is close to this 20,000–60,000 cells/ml band.

It is unlikely that elevation of SCC is the only method by which quarters infected with *C. bovis* are protected. Schukken and colleagues concluded that because SCC was included in the logistic regression model used to analyse their results, the protective effect conferred by *C. bovis* infection was partly independent of SCC (38). In another study, Lam and colleagues demonstrated that IMI with CNS elevated quarter SCC more, but infection was associated with less protective affect. They concluded that the protective effect of *C. bovis* is likely to be due to other mechanisms as well as an increase in SCC (27).

C. bovis infections may protect quarters by interfering with the ability of other pathogens to invade or multiply within the quarter. Bacteria can produce a wide range of molecules and substances that can inhibit the growth of and in some circumstances kill other bacteria (25).

The most widely studied inhibitory factors produced by bacteria are bacteriocins. Bacteriocins are bactericidal protein containing molecules produced by some species of bacteria (25, 40). Many members of the Corynebacterium genus have been shown to produce bacteriocins (1, 10, 26). Recently, *C. bovis* was shown to produce a bacteriocin that demonstrated inhibitory activity against *Listeria monocytogenes* (10).

GENERAL MATERIALS & METHODS

As part of a dry cow intervention study 505 cows from 16 herds in SW England were aseptically quarter sampled for bacteriological analysis at three time points (drying off, calving and 7-14 days after calving). In addition samples were collected aseptically from all cows that suffered clinical mastitis over a 12-month period. Animals were selected for inclusion in the study if all routine cow SCC were ≤ 200,000 cells/ml and the cows had no cases of clinical mastitis during the preceding lactation. Some 252 animals received an internal teat sealant based on bismuth sub-nitrate (Orbeseal, Pfizer Animal Health) and 253 received a long acting antibiotic tube containing cephalonium (Cepravin Dry Cow, Schering-Plough Animal Health).

DIFFERENTIATION OF C. bovis FROM OTHER CORYNEBACTERIUM SPECIES

INTRODUCTION

Lipophilic coryneform species of mammary gland origin are often assumed to be *C. bovis*. *C. bovis* can be differentiated from other lipophilic species based on sugar fermentation profiles and enzymic reactions (15). Two commercial identification kits based on these reactions are available (API Coryne and the Biolog system), however they correctly identified only 88.0% and 54.0% of *C. bovis* isolates in a recent study (42). 16S rRNA gene sequencing is a recognized "gold standard" for the speciation of bacteria. However, this technique is both expensive and time consuming, and still out-with the capabilities of most laboratories. Enzyme restriction and agarose gel visualization of the 16S rRNA gene sequence to produce species-specific restriction patterns is a simpler technique and may offer a viable alternative to gene sequencing.

MATERIALS & METHODS

Bacterial isolates collected during the study were identified as coryneforms by standard bacteriological techniques. Lipophilic and non-lipophilic species were differentiated based on their growth pattern on brain heart infusion agar with or without the presence of free fatty acid added to the growth media (Tween 80, one percent v/v).

A corynebacterial 16S rRNA nucleotide sequence database was created from published data. Specific enzyme restriction patterns were predicted from analysis of the database, for all lipophilic species. It was predicted that endonuclease restriction with *Hind* III and *Sma* I would differentiate *C. bovis* from all other lipophilic species.

Template DNA was extracted from all lipophilic isolates by boiling and the 16S rRNA region was amplified by polymerase chain reaction. Resulting PCR products were purified, cleaved using *Hind* III and *Sma* I restriction enzymes and visualized by 1% agarose gel electrophoresis. Restriction patterns were compared to database predictions to allow species identification.

PRELIMINARY RESULTS & CONCLUSIONS

Coryneform bacteria were isolated from 934 of 5878 (15.9%) screening samples and from 50 of 534 (9.4%) mastitis samples. Lipophilic species accounted for 763 of the 934 (81.7%) screening and 42 of the 49 (85.7%) clinical mastitis sample coryneforms.

781 lipophilic isolates from screening and mastitis samples had *Hind* III and *Sma* I restriction profiles consistent with data base predictions specific for *C. bovis*, equivalent to 97.0% of lipophilic and 79.4% of all coryneform isolates. 24 coryneform isolates (22 from screening and 2 from mastitis samples) had *Hind* III and *Sma* I restriction profiles that were inconsistent with *C. bovis*.

Our preliminary results suggest that it is not safe to assume that all lipophilic coryneforms isolated from milk or mastitis samples are *C. bovis*, 3% (24 in 805) were identified as species other than *C. bovis* in this study. For routine clinical purposes this error is of little consequence, however for epidemiological studies and investigations of "*C. bovis* mastitis" outbreaks the assumption that lipophilic coryneforms of milk origin are *C. bovis* should be made with care.

PREVALENCE AND SIGNIFICANCE OF C. bovis IN AND AROUND THE DRY PERIOD

MATERIALS & METHODS

Following differentiation of *C. bovis* from other Corynebacterium species using the method outlined above, the data set collected during the study was analysed using univariate and multivariate statistical methods to assess the significance of *C. bovis* infection in and around the dry period.

PRELIMINARY RESULTS

The overall prevalence of *C. bovis* in cows with SCC ≤ 200,000 cells/ml in the 16 herds was 21.2% (429 of 2020), 38.0% (192 of 505) and 81.3% (13 of 16) at the quarter, cow and farm levels respectively, at drying off. On individual farms the prevalence ranged from 0% to 74% at the quarter level and 0% to 100% at the cow level. The four farms with the lowest prevalence of *C. bovis* at the quarter level had the 1st, 10th, 14th and 15th highest BMSCC; the four farms with highest prevalence had the 2nd, 8th, 11th and 16th highest BMSCC.

The affect of *C. bovis* infection on SCC is outlined in Table 1. Quarters infected with *C. bovis* only had significantly higher SCC compared to quarters that yielded no growth at all three sampling time points.

Table 1   Somatic cell count of quarters infected with *C. bovis* and those yielding no growth at three sampling time points

Parameter	Drying Off		Calving		Post-calving	
	n	SCC[1]	n	SCC[1]	n	SCC[1]
C. bovis[2]	363	457.1[a]	69	933.3[a]	173	208.9[a]
No Growth	1112	173.8[b]	917	371.5[b]	1337	45.7[b]

Numbers within columns with different superscripts differ, $p < 0.05$.
[1]Geometric mean SCC x1000 cells/ml.
[2]Quarters yielding *C. bovis* only and no other pathogen

The overall dry period cure rate (*C. bovis* positive in drying off samples and negative in calving samples) of *C. bovis* IMI was 83.0%. It was, however, significantly higher in the group that received antibiotic DCT compared to those that received the teat sealer (99.5%, 210 of 211 compared to 67.0%, 146 of 218, *p*<0.001). The new *C. bovis* dry period IMI rate was significantly higher in the teat seal group compared to the group that received antibiotic DCT (4.0%, 30 of 752 quarters at risk *cf*. 0%, 0 in 765 quarters at risk, *p*<0.001).

Compared to quarters not infected with *C. bovis*, quarters that were *C. bovis* positive at drying off, calving and 7–14 days after calving were significantly less likely to be concurrently infected with a major mastitis pathogen (univariate analysis). Compared to quarters that were *C. bovis* negative at drying off and calving, quarters that retained a *C. bovis* IMI during the dry period (culture positive at drying off and calving) were significantly less likely to acquire a new dry period IMI caused by a major pathogen (Multivariate analysis, *p*=0.04).

DISCUSSION

Quarters infected with *C. bovis* had a significantly higher SCC than quarters that were bacteriologically negative. This was true at all three sampling time points and is in agreement with other authors. Elevations is SCC may have been higher in this study because SCC tends to be higher at drying off and around calving (2) and samples were collected in the morning shortly after milking; residual strippings from quarters have higher counts (22).

The prevalence of *C. bovis* between farms showed huge variation at both the quarter and cow levels even in cows with a history of low SCC. There also seems no obvious link between BMSCC and prevalence of *C. bovis*. This is in agreement with data presented by Hillerton and colleagues that demonstrated on one farm where the prevalence of *C. bovis* increased from less than 30% to greater than 70% over a five year period there was no increase in the BMSCC [20]. One study farm had a quarter and cow level prevalence of 74 and 100%, yet their BMSCC was well below the national average (117,000 cells/ml). It would therefore appear possible to have a very high prevalence of *C. bovis* yet still maintain BMSCC within acceptable limits.

Our results indicate that quarters infected with *C. bovis* were significantly less likely to be concurrently infected with other pathogens at all three sampling time points (drying off, calving and 7–14 days after calving). Quarters that retained a *C. bovis* IMI during the dry period were significantly less likely to acquire a new dry period IMI caused by a major pathogen. The difference was significant after both univariate and multivariate statistical analysis. Taken as a whole these results would suggest that in this study, quarters infected with *C. bovis* were "protected" against infection with a wide range of other major and minor pathogens. This study, therefore, agrees with the results of other natural infection studies (4, 27, 33, 34, 44). It is also the first to demonstrate that quarters newly infected with *C. bovis* during the dry period are significantly less likely to become infected with other pathogens. The only other studies that investigated the effect of *C. bovis* at this time did not show any statistically significant difference in the new dry period IMI rate (3, 32) and a third only demonstrated protection against clinical mastitis in the following lactation (17).

Elucidating any potentially protective effect that IMI with *C. bovis* may offer is undoubtedly a very complex process because of the many interactions that take place in any complex biological system such as this. Correctly identifying isolates as *C. bovis* and adequately controlling for the effects of confounding factors remains difficult and has almost certainly played a role in explaining some of the apparently conflicting results that the literature describes.

Further work is ongoing at the University of Bristol to identify the method or methods by which *C. bovis* "protects" quarters. Initial results would suggest that *C. bovis* might be producing a factor which is inhibitory to other pathogens, a possibility previously suggested by Black and colleagues (4).

CONCLUSIONS

C. bovis is one of the most prevalent pathogens of the bovine mammary gland. It is undoubtedly association with a small elevation in SCC and on occasions may be association with clinical disease. There is, however, increasing evidence to suggest that quarters infected with *C. bovis* are protected against infection with other more pathogenic mastitis organisms. The results described here report the first study to demonstrate that quarters infected with *C. bovis* during the dry period are less prone to subsequent IMI with other pathogens at that time. The identity of *C. bovis* was confirmed by analysis of the 16S rRNA gene sequence and the results were analysed using a logistic regression model. The mild elevation in SCC associated with infection with *C. bovis* may be a price worth paying to protect quarters against more serious disease. In the future it may be possible to identify the mechanisms by which *C. bovis* confers protection and utilise them to prevent infection with pathogenic mastitis organisms.

ACKNOWLEDGEMENTS

REFERENCES

1. Abrehem, K. and Zamiri, I. (1983) Production of a bacteriocin, Ulceracin-378, by *Corynebacterium ulcerans. Antimicrob. Agents Chemother.* **24**: 262-267.
2. Barkema, H.W., Deluyker, H.A., Schukken, Y.H. and Lam, T.J. (1999) Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Prev. Vet. Med.* **38**: 1-9.
3. Berry, E.A. and Hillerton, J.E. (2002) The effect of selective dry cow treatment on new intramammary infections. *J. Dairy Sci.* **85**: 112-121.
4. Black, R.T., Bourland, C.T. and Marshall, R.T. (1972) California Mastitis Test reactivity and bacterial invasions in quarters infected with *Corynebacterium bovis. J. Dairy Sci.* **55**: 1016-7.
5. Black, R.T., Marshall, R.T. and Bourland, C.T. (1972) Locus of mammary gland infections of *Corynebacterium bovis. J. Dairy Sci.* **55**: 413-6.

6.   Bramley, A.J., Kingwill, R.G., Griffin, T.K. and Simpkin, D.L.   (1976) Prevalence of *Corynebacterium bovis* in bovine milk samples. *Vet. Rec.* **99**: 275.

7.   Brooks, B.W. and Barnum, D.A. (1984) Experimental colonization of the bovine teat duct with *Corynebacterium bovis* and the effect on milk somatic cell counts. *Can. J. Comp. Med.* **48**: 141-5.

8.   Brooks, B.W. and Barnum, D.A. (1984) The susceptibility of bovine udder quarters colonized with *Corynebacterium bovis* to experimental infection with *Staphylococcus aureus* or *Streptococcus agalactiae. Can. J. Comp. Med.* **48**: 146-50.

9.   Brooks, B.W., Barnum, D.A. and Meek, A.H. (1983) An observational study of *Corynebacterium bovis* in selected Ontario dairy herds. *Can. J. Comp. Med.* **47**: 73-8.

10. Carnio, M.C., Eppert, I. and Scherer, S. (1999) Analysis of the bacterial surface ripening flora of German and French smeared cheeses with respect to their anti-listerial potential. *Int. J. Food Microbiol.* **47**: 89-97.

11. Cobb, R.W. and Walley, J.K. (1962) *Corynebacterium bovis* as a probable cause of bovine mastitis. *Vet. Rec.* **74**: 101-102.

12. Corbeil, L.B., Woodward, W., Ward, A.C.S., Mickelsen, W.D. and Paisley, L. (1985) Bacterial Interactions in Bovine Respiratory and Reproductive Infections. *J. Clin. Microbiol.* **21**: 803-807.

13. Counter, D.E. (1981) Outbreak of bovine mastitis associated with *Corynebacterium bovis. Vet. Rec.* **108**: 560-1.

14. Doane, R.M., Oliver, S.P., Walker, R.D. and Shull, E.P. (1987) Experimental infection of lactating bovine mammary glands with *Streptococcus uberis* in quarters colonized by *Corynebacterium bovis. Am. J. Vet. Res.* **48**: 749-54.

15. Funke, G., von Graevenitz, A., Clarridge 3rd, J.E. and Bernard, K.A. (1997) Clinical microbiology of coryneform bacteria. *Clin. Microbiol. Rev.* **10**: 125-59.

16. Green, L.E. (2000) Latest situation on low SCC and subsequent intramammary infection. *Cattle Practice* **8**: 239-241.

17. Green, M.J., Green, L.E., Medley, G.F., Schukken, Y.H. and Bradley, A.J. (2003) Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *Submitted for publication.*

18. Harmon, R.J., Crist, W.L., Hemken, R.W. and Langlois, B.E. (1986) Prevalence of minor udder pathogens after intramammary dry treatment. *J. Dairy Sci.* **69**: 843-9.

19. Hassan, Z., Daniel, R.C., O'Boyle, D. and Frost, A.J. (1999) Effects of dry cow intramammary therapy on quarter infections in the dry period. *Vet. Rec.* **145**: 635-9.

20. Hillerton, J.E., Staker, R.T. and Shearn, M.F. (1995) Failure of exit-race teat spraying to control *Corynebacterium bovis* colonisation. *Vet. Rec.* **137**: 633-5.

21. Hogan, J.S., Smith, K.L., Todhunter, D.A. and Schoenberger, P.S. (1988) Rate of environmental mastitis in quarters infected with *Corynebacterium bovis* and *Staphylococcus* species. *J. Dairy Sci.* **71**: 2520-5.

22.   Holdaway, R.J., Holmes, C.W. and Steffert, I.J. (1996) A comparison of indirect methods for diagnosis of subclinical intramammary infection in lactation dairy cows. *Aust. J. Dairy Tech.* **51**: 64-71.

23.   Hommez, J., Devriese, L.A., Vaneechoutte, M., Riegel, P., Butaye, P. and Haesebrouck, F. (1999) Identification of non-lipophilic corynebacteria isolated from dairy cows with mastitis. *J. Clin. Microbiol.* **37**: 954-7.

24.   Honkanen-Buzalski, T., Griffin, T.K. and Dodd, F.H. (1984) Observations on *Corynebacterium bovis* infection of the bovine mammary gland. I. Natural infection. *J. Dairy Res.* **51**: 371-8.

25.   Jack, R.W., Tagg, J.R. and Bibek, R. (1995) Bacteriocins of gram-positive bacteria. *Microbiol. Rev.* **59**: 171-200.

26.   Kato, F., Yoshimi, M., Araki, K., Motomura, Y., Matsufune, Y., Nobunaga, H. and Murata, A. (1984) Screening of Bacteriocins in Amino-Acid or Nucleic-Acid Producing Bacteria and Related Species. *Agric. Biol. Chem.* **48**: 193-200.

27.   Lam, T.J., Schukken, Y.H., van Vliet, J.H., Grommers, F.J., Tielen, M.J. and Brand, A. (1997) Effect of natural infection with minor pathogens on susceptibility to natural infection with major pathogens in the bovine mammary gland. *Am. J. Vet. Res.* **58**: 17-22.

28.   Lam, T.J., van Vliet, J.H., Schukken, Y.H., Grommers, F.J., van Velden-Russcher, A., Barkema, H.W. and Brand, A. (1997) The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds. II. Dynamics of intramammary infections. *Vet. Q.* **19**: 47-53.

29.   Linde, C., Holmberg, O. and Astrom, G. (1980) The interference between coagulase negative staphylococci and *Corynebacterium bovis* and the common udder pathogens in the lactating cow. *Nord. Vet. Med.* **32**: 552-8.

30.   Morin, D.E. and Constable, P.D. (1998) Characteristics of dairy cows during episodes of bacteriologically negative clinical mastitis or mastitis caused by *Corynebacterium spp. J. Am. Vet. Med. Assoc.* **213**: 855-61.

31.   Ngatia, T.A., Jensen, N.E. and Berg, B.B. (1991) Changes in the bovine udder quarters naturally infected by *Corynebacterium bovis. Br. Vet. J.* **147**: 463-8.

32.   Pankey, J.W., Nickerson, S.C., Boddie, R.L. and Hogan, J.S. (1985) Effects of *Corynebacterium bovis* infection on susceptibility to major mastitis pathogens. *J. Dairy Sci.* **68**: 2684-93.

33.   Pociecha, J.Z. (1989) Influence of *Corynebacterium bovis* on constituents of milk and dynamics of mastitis [letter; comment]. *Vet. Rec.* **125**: 628.

34.   Rainard, P. and Poutrel, B. (1988) Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle. *Am. J. Vet. Res.* **49**: 327-9.

35.   Robinson, T.C. and Harwood, D.G. (1998) Mastitis in a dairy herd associated with *Corynebacterium bovis* [letter] [see comments]. *Vet. Rec.* **143**: 147.

36.　Robinson, T.C., Jackson, E.R. and Marr, A. (1983) Within herd comparison of teat dipping and dry cow therapy with only selective dry cow therapy in six herds. *Vet. Rec.* **112**: 315-9.

37.　Schalm, O.W., Lasmanis, J. and Carroll, E.J. (1964) Effects of pre-existing leukocytosis on experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. *Am. J. Vet. Res.* **25**: 83-89.

38.　Schukken, Y.H., Leslie, K.E., Barnum, D.A., Mallard, B.A., Lumsden, J.H., Dick, P.C., Vessie, G.H. and Kehrli, M.E. (1999) Experimental *Staphylococcus aureus* intramammary challenge in late lactation dairy cows: quarter and cow effects determining the probability of infection. *J. Dairy Sci.* **82**: 2393-2401.

39.　Schukken, Y.H., Vanvliet, J., Vandegeer, D. and Grommers, F.J. (1993) A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. *J. Dairy Sci.* **76**: 2925-30.

40.　Tagg, J.R., Dajani, A.S. and Wannamaker, L.W. (1976) Bacteriocins of Gram-positive bacteria. *Bacteriol. Rev.* **40**: 722-756.

41.　Viseslava, M. and Vera, K. (1989) The effect of *Corynebacterium bovis* colonization of the mammary gland of cows on the incidence of *Streptococcus agalactiae* and *Staphylococcus aures* mastitis. *Acta Vet.-Beogr.* **39**: 121-125.

42.　Watts, J.L., Lowery, D.E., Teel, J.F. and Rossbach, S. (2000) Identification of *Corynebacterium bovis* and other coryneforms isolated from bovine mammary glands. *J. Dairy Sci.* **83**: 2373-2379.

43.　Wilson, D.J., Gonzalez, R.N., Han, H.R. and Schukken, Y.H. (2001) Adoption of mastitis control practices, dairy farm characteristics and associations with milk quality. *2nd International Symposium on Mastitis and Milk Quality* Vancouver, BC, Canada.

44.　Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Wellenberg, G.J., Grohn, Y.T. and Schukken, Y.H. (2001) Cow- and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. *J. Dairy Sci.* **84**: 2649-2663.

THE MILKING LINER

D. Boast[1], M. Hale[1], M. Bennett[1] and J.E Hillerton[2]

[1] Avon Rubber, Materials Development Centre, Brook Lane Industrial Estate, Westbury BA13 4EP

[2] Institute for Animal Health, Compton, Newbury, Berks RG20 7NN

SUMMARY

For some time farmers have been advised to change milking liners after 2500 milkings or 6 months. The origin of these recommendations is unclear and their justification is not in the public domain. Future liner development needs as a starting point some clear knowledge of the how the liner affects milking performance. Although much work has been done on the liner design and the development of materials, many of the aspects of liner deterioration and how these affect liner performance were unknown. A series of farm trials were done where the liners were aged and the key dimensions, mechanical properties and chemical changes in the liner were investigated. The milking performance of liners of known age was then compared with the liner properties.

Modelling and laboratory testing of specific liner attributes further added to the understanding of mechanisms of teat damage and changes in the milking performance suggested by the farm trials.

INTRODUCTION

The forces on the cow's teat are only a result of the liner vacuum, milk flow and the forces applied by the rubber during pulsation. These forces will vary as the liner material and shape changes over the liner life. The changes in the liner will affect certain aspects of the liner performance, especially the effect on teat condition. If the teat is overstressed during milking then the teat may be more susceptible to infection.

In addition to the variations in the shape of liner and the set-up of the milking machine, the liner material changes over its life. An understanding of the detailed changes in the liner and milking performance is important to understand the mechanisms of how the properties of the liner affect the teat end condition, and the milking performance. The general conditions of liner use will alter the details of the liner changes, so the conditions will vary from farm to farm.

DETAILS OF FARM TRIALS

A batch of DeLaval 960000-01 liners were fitted into DeLaval HC150 clusters in a double 8 DeLaval milking parlour operating at 47 kPa plant

vacuum and pulsation of 60 pulses min and 60% ratio. A herd of approximately 230 cows was milked twice daily. Approximately every 400-cow milkings one set of liners was removed for study. After 1500 milkings the liners were re-tensioned.

Measurements were taken of teat length and width before milking, but after stimulation, the length of cluster attachment time, the whole udder yield every 30 seconds and teat length and width after milking were also measured. Immediately after cluster removal each teat was scored for colour, response to touch, any obvious deformation distally or proximally, and orifice status (3). Finally the completeness of milking was assessed by the stripping of milk from any teat for longer than 15 seconds.

Average milk flow rate was calculated as the total yield divided by the cluster attachment time. Peak flow rate was calculated from the change in yield from 1 minute to 3 minutes after cluster attachment

All four liners still in their shells were removed to the Avon Rubber test laboratory where key dimensions were measured (1). The liners were then examined by Scanning Electron Microscopy (SEM) and energy dispersive x-ray spectrometry (EDAX) for changes inside the barrel. Gas Chromatography (GC) and Thermogravimetric Analysis (TGA) were used to investigate the composition of the rubber. Dynamic Mechanical Thermal Analysis (DMTA) measured changes in mechanical properties of the rubber.

RESULTS

Details of the liner changes

The main changes that take place in the liner are in the material and the shape of the liner. The liner tension in the shell reduces due to creep and swell, the barrel becomes oval and the mouthpiece distorts. The material absorbs water and butterfat and certain parts of the rubber formulation are extracted from the butter into the milk. The surface of the rubber can become rough from deposit of milkstone.

Two of the main areas of interest are in the forces around the teat end and in the forces on the base of the teat where the mouthpiece of the liner seals. The forces on the teat end are shown in Figure 1. The contact pressures are governed by the basic membrane equation.

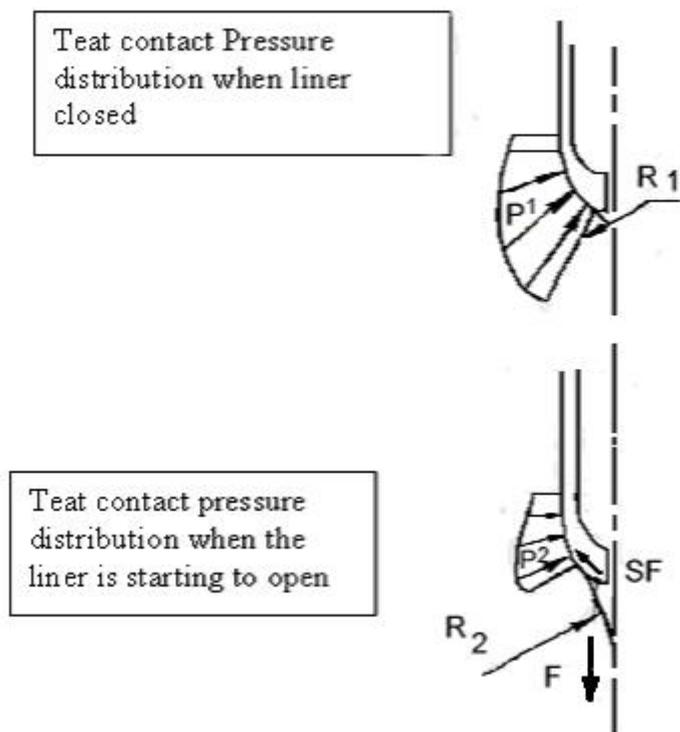$$\text{Tension} = \text{pressure} * \text{radius} \qquad (\text{Equation 1})$$

or

$$\text{Pressure} = \text{tension} / \text{radius} \qquad (\text{Equation 2})$$

Thus the contact pressure can be calculated from knowledge of the tension and radius. The contact pressure with the teat will vary as the contact

radius changes (P_1 and P_2). The force F is due to the vacuum. The force marked SF is a shear force between the liner and the teat.

Figure 1   Teat contact pressures

Butterfat and water change the rubber. The mechanism of how the butterfat gets into the rubber is important as this explains the localised changes in the rubber. The action of the rubber closing on the teat causes mechanical attrition of the milk. The butterfat is forced out of the emulsion and some is deposited on the liner surface. This results in uneven deposition of butterfat on the surface and an uneven change in properties in the liner. The high temperatures during wash dramatically increase the uptake of butterfat. The liner locally stiffens as the age increases but only in the area in contact with the cow's teat.

Rubber creep causes relaxation of the tension in the barrel, which, in turn reduces the contact forces of the rubber against the teat (see equation 2). As the liner creeps the reduction in tension can result in inadequate massage of the teat resulting in redness, oedema or haemorrhaging. Swell of the rubber due to the absorption of water and butterfat reduces the barrel tension. High temperatures during wash also accelerate the creep of the rubber.

The milk deposited onto the liner surface is responsible for the build up of milkstone locally, and the change in depth and character of the surface down the liner barrel. The rough milkstone layer on the liner barrel
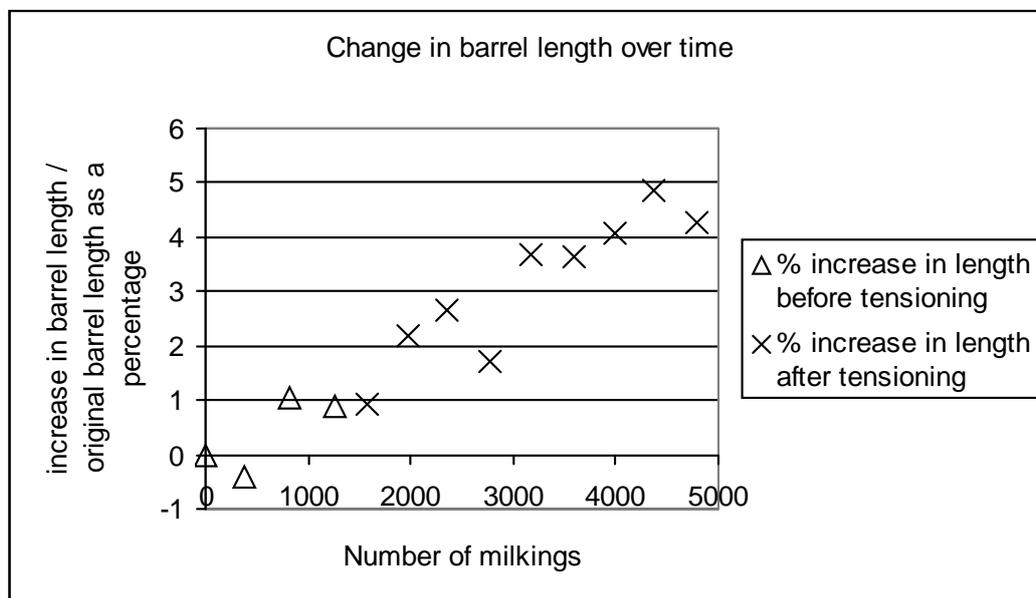
increases the friction and the teat damage caused when the liner closes on the teat.

Some mouthpiece distortion occurs due to milking but much of the mouthpiece distortion will occur during washing. The high temperatures during washing accelerate the rate of distortion.

Barrel length and tension

The increase in barrel length is shown in Figure 2. This causes a significant reduction in the tension and therefore the teat contact pressure.
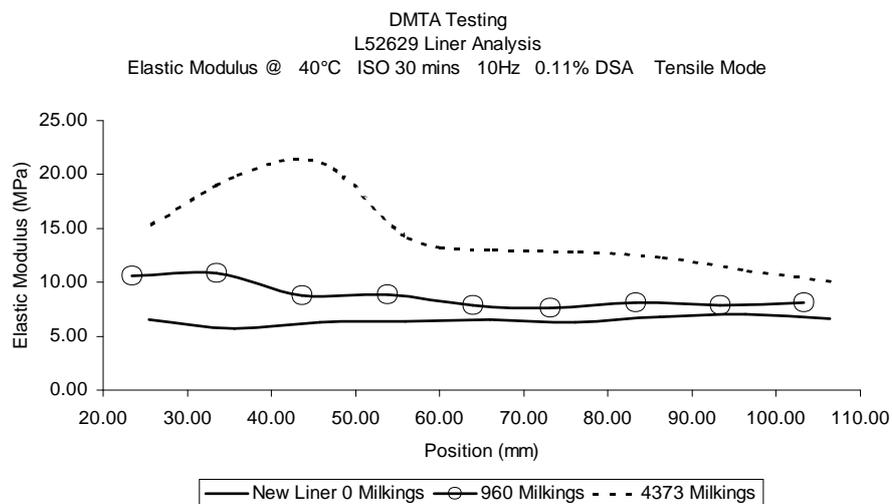
Figure 2   Change in barrel length

As the rubber is stretched then the barrel diameter reduces. This imposes a limit on the amount the liner can be re-tensioned.

Figure 3 shows the localised changes due to the chemical changes in the bulk rubber and the surface of the liner. The older liner becomes 3 times stiffer in the teat end contact region, (The elastic modulus is roughly equivalent to Young's modulus).

Figure 3   Localised change in liner stiffness down the liner barrel

DMTA Testing
L52629 Liner Analysis
Elastic Modulus @   40°C   ISO 30 mins   10Hz   0.11% DSA   Tensile Mode

Legend: —— New Liner 0 Milkings —⊖— 960 Milkings - - - 4373 Milkings

Deposits on the surface of the liner

Liners often have a form a textured surface due to a deposit of calcium and phosphorous. This is often called milkstone. We believe that the deposit is sometimes wrongly diagnosed as some sort of surface degradation and erosion of the rubber, generally attributed to the cleaning regime.

Figure 4   Textured surface of the milkstone calcium/phosphorous deposit on the liner. Magnification approx x200.

The milkstone layer is rough, has high friction and stiffens the liner. The build up is local due to the uneven deposition of the butterfat.

Changes to the cow as liners age

Figure 5 shows that as the liner ages the proportion of teats appearing red or blue on cluster removal increases.

Figure 5   Teat colour changes as liners age
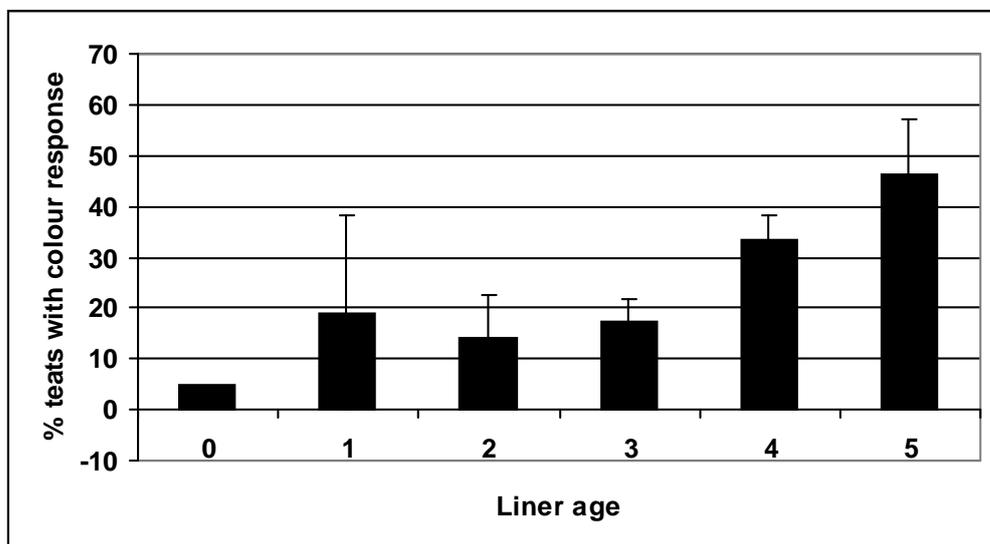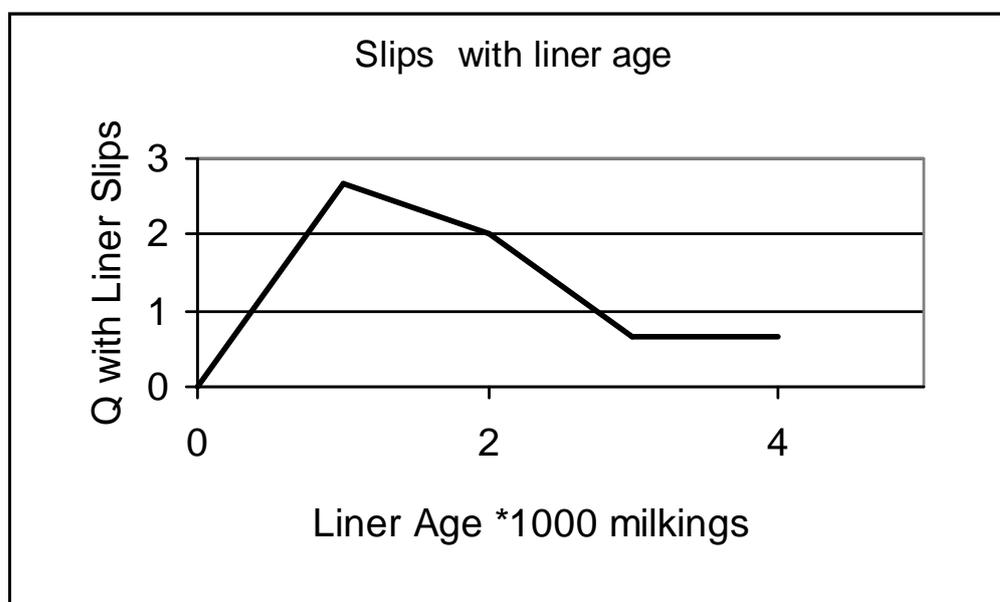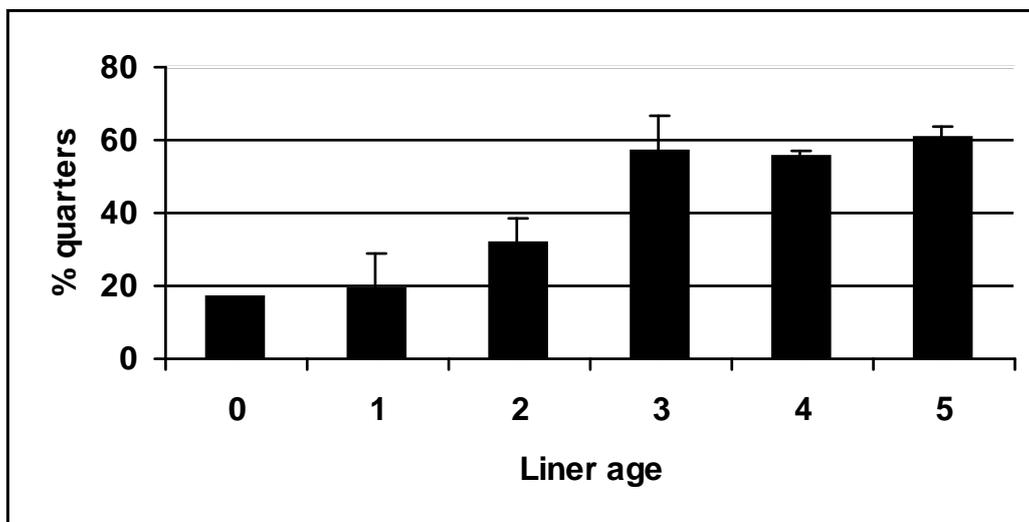
Figure 6   Change in liner slips as liners age
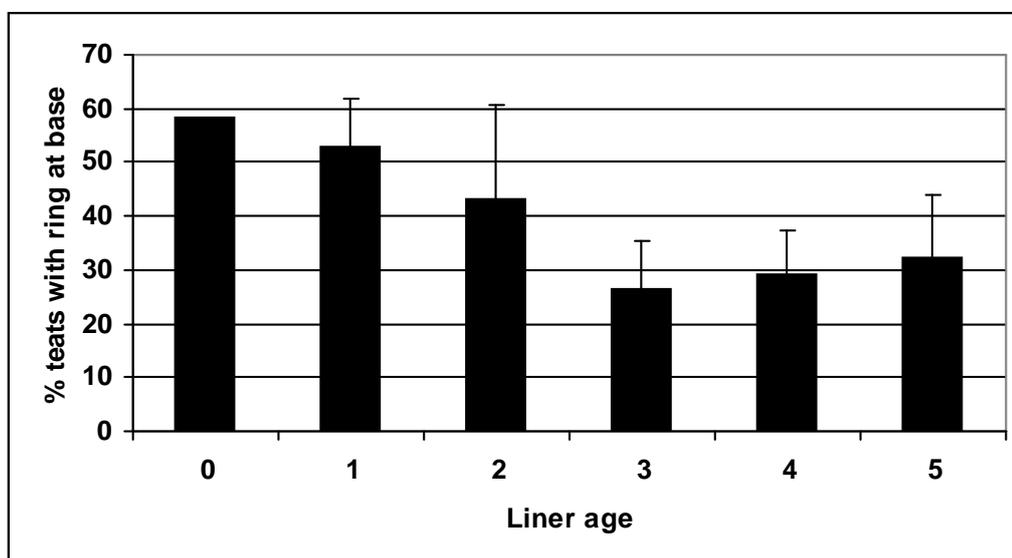
The number of liner slips (Figure 6) in our tests increased and then decreased. The increase with age correlates with the change in friction from the milkstone build up. The increased friction will probably stress the teat more.

Figure 7   Change in the strip yield as the liners age

The strip yield increased significantly even before the recommended change time of 2500 milkings.

Figure 8   Change in the incidence of ringing at the base of the teat

The material changes that take place in the liner mouthpiece give some reduction in the ringing at the base of the cow's teat. This shows the some of the compromises that may be needed in liner design.

DISCUSSION

The changes that take place in the liner are significant in a correctly functioning milking parlour. However, the changes in the liner will become more critical if the milking machine is not working correctly. Liners can take up to 0.25 seconds longer to open and close, depending on the set-up of the milking parlour (4).

In the near future changes to the regulations governing the ingredients that rubber makers can use to make liners will result in the withdrawal of various ingredients that help to prolong the liner life. As a result liners will degrade more quickly and changing the liner at the prescribed intervals will become more important.

As the liner deteriorates then the teat massage becomes increasingly ineffective and the teats are subject to more mechanical stress. The mechanism of the elastic liner closing round the elastic teat is complex. However, our determination of the key liner material characteristics forms the basis for mathematical models developed to investigate the various teat end damage mechanisms. These models are currently being verified and will then be used to guide the development of liners.

The period of use of liners, 2500 milkings or 6 months, will encompass a wide range of exposure to factors that causes deterioration of the liner. Wash frequency will be the same in small and large herd sizes. The high temperature of the wash probably dominates some of the deterioration processes and especially in a small herd the liners will receive more washes. Rubber is sensitive to a temperature change as small as 10°C. This will cause a significant change in many of the rubber properties described earlier e.g. the permeation of butterfat is increased 10 times when the temperature is raised from 20°C to 35°C.

CONCLUSIONS

A clear understanding of the changes that take place in a liner confirms that the liners should be changed at 2500 milkings. Various factors reduce the tension and therefore the pressure of the rubber on the teat. The changes that take place in the liner are localised. This means that any testing of liner rubber is done with careful regard to sample position and sample size. The effectiveness of the massage is reduced as the liners age. Indicators of the teat condition, such as the teat discolouration and strip yield, show the teat is becoming more highly stressed as the liner ages.

ACKNOWLEDGEMENTS

REFERENCES

1.   Boast, D., Hale, M., Hillerton, J.E., Middleton, N. and Ohnstad, I. (2003) Variation in the rubber chemistry and dynamic mechanical properties as liners age. *IDF World Dairy Summit Conference on "100 years with liners and pulsators".* Bruges 2003.

2.   Hillerton, J.E., Boast, D., Davies, D., Middleton, N. and Ohnstad, I. (2003) Changes in milking liner performance with age*. Proc. 42nd Nat. Mastitis Council Annual Meeting,* Fort Worth USA, pp 239-250.

3.   Hillerton, J.E., Ohnstad, I., Baines, J.R. and Leach, K.A. (2000) Changes in cow teat tissue created by two types of milking cluster. *J. Dairy Res.* **67:** 309-317.

4.   Spencer, S.B. and Rogers, G.W. (2003) Optimization of a milking machine liner*. Proc.42nd Nat. Mastitis Council Annual Meeting,* Fort Worth USA, pp 234-238.

A STUDY INTO MILKING MACHINE AUTOMATION

Brian R. Pocknee

ADAS Dairy Group, Ceres House, 2 Searby Road, Lincoln, Lincolnshire LN2 4DW

SUMMARY

The aim of a modern milking parlour is to milk cows efficiently, safely, with due regard to the welfare and behaviour of the cow and to satisfy all milk hygiene requirements. An MDC funded study of new and proposed investments in milk harvesting equipment in the UK provided benchmarks for the development of a decision-making tool (New Parlour Wizard). This allows farmers to assess the effectiveness of the existing parlour and to make informed decisions when investing in new milking systems and facilities. The Wizard calculates milking time, investment costs and the financial affects on the dairy farming business of the proposed investment.

Milk quality and udder health can be seriously compromised if the type and size of the parlour does not allow for an efficient and effective milking routine. Although the New Parlour Wizard is an economic appraisal tool, it also allows the user to see the impact of beneficial automation on parlour performance. Often the main benefit of automation is that it releases time for essential elements of the milking routine to be retained without compromising parlour throughput.

INTRODUCTION

The main objectives of parlour milking are to milk cows as efficiently, gently and safely as possible while complying with all current milk hygiene standards (6, 9). However, falling milk prices have eroded financial margins and increased the trend towards larger herds. The pool of qualified personnel to milk and manage has also reduced.

These changes have often resulted in a conflict between individual cow attention and speed of milking (10, 13), leading farmers to seek alternative, often larger milking systems to try and balance the time spent in the parlour with time for other duties. These introduce dangers for udder health and cow welfare to be compromised.

In the UK there are 7 main types of milking parlour:

- ➢ Abreast
- ➢ 1:2 herringbone (one milking unit shared between two cow standings)
- ➢ 1:1 herringbone (one milking unit for each cow standing)

> ➢ Rapid exit (cows stand at 90 degrees to the operator and exit out of the side of the parlour)

> ➢ Auto tandem (one unit per individual cow standing)

> ➢ Rotary parlour (operator standing either inside or outside the platform)

> ➢ Automatic milking system (AMS)

Each has benefits and weaknesses, and in the UK one of these options can allow the individual farmer's specific requirements to be broadly met. Inevitably, however, there has to be a trade-off between the ideal, capital available and future business viability.

With this in mind there is the need for the industry to recognise the impact that the milking routine has on parlour performance, and that reducing the thoroughness of any element of the routine or eliminating certain elements is likely to impinge on udder health. One of the reasons for the recent increase in bulk tank milk SCC has been attributed to cuts in critical elements in the milking routine (7). However, automation, if correctly implemented, can provide positive responses.

The type of parlour is only one part of the equation when considering milking performance (5, 4). In almost all cases the throughput of any parlour is dictated by the milking routine.

The correct implementation of automation linked to milk harvesting should release time for essential elements of the work routine [as originally promoted in the NIRD/CVL Five Point Plan and more latterly the DEFRA Mastitis Management Action Plan] to be performed effectively. When a full milking routine is practised the speed of throughput can only be improved by increasing the speed of entry into and exit out of the parlour and the level of automation in the parlour, such as ACR and auto identification.

The study reviewed existing parlour automation, together with technology for the future, some of which has been presented to previous British Mastitis Conferences. However, the principal aim of the study was a survey of new parlour installations and the development of a decision-making model (New Parlour Wizard) to be used by the industry. The model allows a farmer to review the existing facilities and provides a cost-benefit appraisal of the parlour options available, based on criteria selected by the producer.

This paper concentrates on the milk quality and udder health issues that relate to parlour investment.

SURVEY

In 2002 and 2003 a letter was sent out to producers who had recently installed new parlours. The survey sought detailed information both pre- and post-installation including on herd size, milk production data (yields, composition and hygiene quality), farm details, labour use and parlour information including size, amount and type of automation, milking duration and milking routines/methods. An estimate of total costs split between parlour equipment and fittings, and structural (building) works was also requested. Finally, farmers were asked to identify the benefits of the new installation to their business and where they would have made different decisions if they were to repeat the process.

From the details collected a model, known as the New Parlour Wizard, was developed for use by the industry. It comprises two distinct sections, with the first dealing with the existing farm situation and the second with the proposed investment. Further details are available from www.mdc.org.uk

RESULTS AND DISCUSSION

A response rate of 27% was achieved, with 47 detailed returns analysed.

The vast majority of new parlours were "swing over" herringbones, with an average configuration of 15:30 . Of the 1 unit:1 stall parlours, herringbones were also the most popular with an average size of 22:22. As a generalisation to achieve high throughputs, larger parlours are generally required. However, there were a number of exceptions to this rule.

Capital investment

The average investment was £99,658, with the building to parlour cost ratio of 45:65. The average cost was £5,733 per cow standing, £601 per cow and 9 p/quota litre. The total cost equates to a depreciated cost of 0.75 p/litre (building cost depreciated over 15 years and parlour equipment over 10 years).

Reasons for investment

The results of the survey are summarised in Table 1. The respondents gave two main reasons average for their investments. Although udder health and animal welfare are major elements of the many and varied quality assurance schemes, and are high on the agenda of the public and governments, they came low down on the list of producer priorities. The most common reasons for the investment were to replace a worn out parlour and to improve labour efficiency. It is probable that one outcome of the former reason would be to improve udder health and cow welfare.

Interestingly, when it came to seeking advice on the investment in milking facilities, the primary choice for farmers was to ask the parlour manufacturers, i.e. those selling the installation (46%), followed by the banks (25%) and consultants (15%).

Table 1   Reasons for new milking parlour investment

Reason	Number	%
Replace worn out parlour	29	30
To increase labour efficiency	28	29
To facilitate herd expansion	16	17
To improve working conditions	13	14
Improve udder health	3	3
To facilitate change in farming	2	2
To have option of third milking	1	1
To meet hygiene requirements	1	1
Greenfield site	1	1
Improve management information	1	1
New holding	1	1
TOTAL	96	100

Actual benefits from investment

On average the respondents gave more than two benefits of the investment (Table 2). Although improved labour efficiency and working environment were the most important benefits as viewed by producers, improvements in udder health and cow welfare appeared of greater importance than in the planning stages. These probably arose due to the elimination of deficiencies in the old milking facilities, i.e. they were a spin-off.

Parlour size and level of automation

There is a good relationship between the size of the new parlour size and the size of the herd, with on average 6–7 standings for every 100 cows. Although less strong, there is on average 10 standings per 500,000 litres of milk output. However, there is no relationship between parlour size and factors such as the number of dairy staff and the level of automation (including ACR, automatic cow identification, backing gates, automatic separation and automatic plant cleaning).

Table 2   Main benefits from investing in a new parlour

Reason	Number	%
Labour efficiency	36	35
Better working environment	30	29
Better management information	13	13
Improved udder health	9	9
Improved cow welfare	6	6
None	3	3
More time off	1	1
Satisfy regulations	1	1
Capacity to increase herd	1	1
Higher yields	1	1
Easier to get relief staff	1	1
TOTAL	102	100

Farm position after investment

Although herd size increased by 17% to 182 cows, parlour performance increased by 64% to 79 cows per hour with the total time spent associated with milking falling by 17% to 2.35 hours. Perhaps a better indicator of parlour performance is the amount of milk harvested per hour. This increased by 68% to 1512 litres. In spite of common assumptions to the contrary, the bulk milk Bactoscan and Somatic Cell Count (SCC) averages both fell (Table 3). This was against a background increase in the national SCC (7).

It is common for dairy farmers to report that the performance of a newly installed or upgraded milking parlour, often does not meet expectations. This has usually been due to a combination of factors. These include overzealous selling of a parlour, a farmer's lack of understanding of what the technology can deliver, inadequate financial provisions leading to essential components (necessary to optimise parlour and milking efficiency) not being installed, or the outcome being examined in isolation, rather than as part of the milk production and harvesting process, e.g. cow flow and behaviour have not been considered.

Table 3   Affects on the herd performance of investment in new milking facilities

	Pre	**Post**	**% change**
Number of dairy staff	2.3	2.3	0
Cows per labour unit	75	86	+15
Herd size	156	182	+17
Milking time (hours)	3.2	2.35	-27
Cows milked per hour	48	79	+64
Annual milk sales ('000 litres)	1,070	1,290	+20
Litres per hour	899	1512	+68
SCC	165	160	-3
Bactoscan	30	28	-7
Yield per cow (litres)	6786	7069	+4

In this study 41% of respondents were satisfied with their parlour, although the remainder would have approached matters differently with hindsight.

Benefits of automation

If used correctly automation releases time for essential elements of the work routine to be completely and effectively performed. This will benefit milk quality, udder health and cow welfare. Milk harvesting should not be seen as a task to be carried out as quickly as possible without due regard to these three issues.

Automatic Cluster Removers (ACR)

If adjusted correctly, an ACR will prevent potentially damaging over-milking (8) and relieve the operator of the task of removing a cluster manually. ACR remove milking clusters from the cow when pre-set milk flow conditions are met, usually around 0.2 kg/min. Recent research has suggested that decreasing the delay time (the time between the minimum flow rate is reached and the cluster is detached, usually around 20-25 seconds) and increasing the minimum flow rate to around 0.4 kg/min has resulted in significant time savings without a loss of production (11). This should only be considered where thorough pre-milking teat stimulation is carried out (1) and will only increase milking speed if the milking duration is currently a major limiting factor in milking efficiency. The benefits of ACR are:

➢   A reduction in the level of over milking of cows, which has been shown to increase the likelihood of teat damage.

➢   Automatic removal of the clusters when milk flow has fallen below a pre-set level can save around 10 seconds per cow. This is worth around 16 minutes each milking for a 100 cow herd.

➢   Potentially higher yields due to lower mastitis and better teat condition.

Backing gates

There are numerous designs for backing gates, some of which do not meet animal welfare considerations (3)! As herd size increases, or where different management groups are maintained, a lifting, backing-gate to separate groups becomes essential. It should be possible to operate the backing gate from various positions within the parlour and the gate must be fitted with a fail-safe mechanism to prevent accidental injury to cows.

Incorrect or aggressive use of the gate can cause some animals to become distressed which can impair the milk let down reflex and slow the milk flow rate (12). The potential benefits include:

➢   Ability to segregate different groups of cows in the collecting yard.

➢   If the backing gate is capable of being lifted, the parlour can be fed continuously with cows, irrespective of the number of group changes.

➢   Maximises time spent on essential milking routines.

➢   Improves cow flow and reduces overall milking time. A well designed backing gate, operating in a well laid out collecting yard, can save at least 10 seconds per cow or 16 minutes per 100 cows.

Automatic identification

The overall benefit of automatic cow identification is that it speeds up milking time when feeding or milk recording. It may add management advantages which can also benefit udder health and milk quality, such as automatic recording of milk conductivity.

Automatic separation

Automatic shedding can save time and reduce the stress of separating cows from the main herd. This can be important, particularly for cows about to be served.

If the milking facility is within an existing building where space is at a premium, auto separation can allow the shedding/holding and handling facility to be located remotely from the milking parlour. This, therefore,

reduces the likelihood of installing an undersized parlour, or having inadequate space for cow exits and dispersal areas which slow down milking.

Milk recording

Electronic milk meters are now common in direct to pipeline parlours. As well as recording milk yield, they control and monitor many of the operations in the parlour and can alert the operator various animal problems and milking machine performance. The milk quality benefits, particularly of automatic milk recording are:

> ➢ Early indication of disease and other problems improving the likelihood of a successful treatment.
> ➢ Help in the prevention of antibiotic contaminated milk entering bulk tank.
> ➢ Improved management information that can be updated daily.

Automatic washing

The length of a milking session in this study included both milking and plant cleaning. A consistent plant washing procedure is required to maintain good milk hygiene. If the automatic plant washer is adjusted and calibrated correctly, it will provide consistency to the wash routine.

An automatic washing will employ a greater number of cleaning cycles than a manual wash and whilst this will often use more water, the cleaning routine is generally improved. An automatic washing system can:

> ➢ Reduce the length of a milking session by around 10-15 minutes
> ➢ Release time for other duties.

Automatic teat spraying

Effective post-milking disinfection is one of the most valuable tools for controlling mastitis. It was a key element of the NIRD/CVL Five Point Mastitis Control Plan and more recently in the DEFRA Mastitis Management Action Plan (Mastitis MAP) (2).

There are a number of methods available to apply disinfectant to cows teats automatically. These use various technical solutions to automatic teat disinfection, but in practise these systems can provide relatively poor teat coverage. However, if they can be developed to provide adequate teat coverage, they could have a considerable benefit on the labour requirement of rotary parlours.

Milk conductivity

Changes in milk conductivity can indicate an emerging intramammary infection and is, therefore, a tool to assist in the early detection of mastitis. Early identification and treatment can often improve the overall cure rate and therefore improve milking efficiency. However, other factors can influence milk conductivity including genetic differences, milk composition, stage of lactation and reproductive status. Therefore, to be effective milk conductivity for each cow needs to be measured over a period of time and any high recording related to the recent recordings.

Milk conductivity recordings measured on an individual quarter basis (as in some robotic milking systems or quarter claws) may provide a more accurate assessment of the infection status of the animal and minimise the number of false positive alarms. To improve accuracy, the conductivity readings need to be corrected for temperature, milking frequency, milking interval and diurnal variation.

CONCLUSIONS

The New Parlour Wizard provides dairy farmers and their advisors with an opportunity to minimise pit falls and ensure that investments in milking facilities are made wisely, with the implications of financial constraints or ideologies being identified at a very early stage in the process. The system identifies inappropriate parlour selection criteria and permits correct decisions to be made with regards to parlour automation, which allows the farmer's expectations to be met, with due regard taken of milk quality, udder health and cow welfare.

ACKNOWLEDGEMENTS

REFERENCES

1. Bruckmair, R.M. (2001) Milk ejection during machine milking in dairy cows. *Livestock Prod. Sci.* **70**: 121-124
2. DEFRA (1999) Treatment and prevention of mastitis. PB 4661.
3. DEFRA (April 2003) Code of Recommendations for the Welfare of Livestock (Cattle).
4. O'Brien, B., O'Callaghan, E. and Dillion P. (1998) Effect of various milking machine systems and components on free fatty acid levels in milk. *J. Dairy Res.* **65**: 335-339.

5.   O'Callaghan, E.J. (1998) Effects of pulsation characteristics on machine yield, milking time and cluster stability. *Irish J. Agric. Food Res.* **37:** 201-207.

6.   O'Rourke, D. J. (1999) The Milking Machine. *Cattle Practice* **7**: 13-14.

7.   Pocknee, B.R. (2002) Progress in milk quality? *Proc. Brit. Mast. Conf.* pp 81-82.

8.   Rasmussen, M.D., Frimer, E.S. and Decker E.L. (1994) Reverse pressure gradients across the teat canal related to machine milking. *J. Dairy Sci.* **77**: 984-993.

9.   Reinemann, D.J., Rasmussen, M.D. and LeMire, S.D. (2002) Milking performance of dairy cows subjected to electrical current and induced milking machine problems. *Am. Soc. Agric. Eng.* **45**: 833-838.

10.   Smith, J.F., Armstrong, D. V. and Gamroth, M.J. (1996) There are ways to speed up milking. *Hoards Dairyman* pp 393.

11.   Stewart, S., Godden, S., Rapnicki, P., Reid, D., Johnson, A. and Eicker, S. (2002) Effects of automatic cluster remover settings on average milking duration, milk flow and milk yield. *J. Dairy Sci.* **85**: 818-823.

12.   Vladimir, T., Dietter, S. and Wolf-Dieter, K. (2000) Cortisol and ACTH release in dairy cows in response to machine milking after pretreatment with morphine and naloxone. *J. Dairy Res.* **67**: 467-474.

13.   Wilson, D. J., Gonzalez, R.N., Southwick, L.H. and Guard, C. L. (2000) Evaluation of an experimental milking pulsation system for effects on milking and udder health. J. Dairy Sci. **83**: 2004-2007.

ANOTHER REPORT ON THE SEARCH FOR THE HOLY GRAIL – finding the mastitic quarter reliably

J. Eric Hillerton

Institute for Animal Health, Compton, Newbury, Berks RG20 7NN

SUMMARY

The basic need to test for abnormal milk is described. Current tests and several under development to detect variations in milk extending to abnormality are listed. The focus is on the difficulty in accepting new or additional parameters indicative of milk quality and composition. The mindset has to change from the entrenched reliance over 30 years on the now familiar and 'comfortable' cell count.

INTRODUCTION

What used to be my post, now any form of paper or electronic communication, contains with remarkable frequency a message or report describing the invention of a reliable test for mastitis. Probably 70-80% of these 'discoveries' are a simple re-hash of perennial solutions, especially involving milk conductivity, and mostly are from people who have done no homework and often know little about cows, mastitis and dairy farming. Now there appear to be more likely tests being developed so it seems appropriate to review them in context with what we need and what we already have available.

TRADITIONAL TESTS

I might say cynically that decades ago it was only the sick cow that was identified because, from cell count records and the apparent incidence of clinical mastitis, most cows were infected (2). The spotting of mastitis was finding the more severe cases or those most persistent. Really sick cows, especially dead cows due to mastitis, were quite rare in times of lower yields and 'relatively' little mastitis caused by *Escherichia coli* (9 cases/100 cows/year when 150 cases/100 cows/year was the average).

The milker spotted mastitic cows by having time to feel the swollen and hot udder, finding clots in foremilk, noticing the inappetant cow and the cow late to the parlour. In the byre much of this was easy but then it was dark so feeling the kick from the mastitic cow was probably also part of detection! If all else failed the milker used a conductivity test by checking if the milk tasted salty.

MOVING ON

In the first half of the 20th century various studies found that chloride content and electrical conductivity of milk showed abnormality simply (4). Microscopic methods allowed measurement of number and type of cells in milk that was more definitive.

The Whiteside test was a crude laboratory test used until instrumented measurements of cell count became possible (11).

The Whiteside test was further developed in 1957 into a cow-side test becoming the California Mastitis Test or CMT (8). Despite careful description of the problems with the test evaluation and its relative insensitivity it remains available and in common uses today because nothing else quick enough, cheap enough and robust enough has been offered to the milker. An automated, in-line CMT has been launched. It works in AMS and is claimed to detect within the cell count range 100,000 – 5M cells/ml (12).

PROMISES

Changes and variations in milk electrical conductivity can be measured easily cow-side and have proven to be very accurate in experimental use but the systems marketed for parlours and in-line use have failed to inspire the market and the potential users. Mostly this is because of poor software, unfriendly presentation and inaccuracy from whole udder measurements. This is a lesson for all sensor development

NEW TECHNOLOGIES

It is a requirement of all milkers to inspect foremilk and to exclude abnormal milk from the bulk supply (9). Tacit attention to that has been the norm. The development of fully automated milking systems (AMS) has raised the horrific suggestion that less that wholesome milk occasionally get into the bulk supply if a milker is not inspecting all milk on every occasion. The manufacturers of AMS have driven forward technology to ensure detection of abnormalities of milk far in excess to what might be detected, even by the hand-milker let alone in the parlour achieving 150 cows/man/hour.

We will thank AMS for the drive to produce better tests, and probably curse the cost!

Quarter conductivity and yield

A true estimation of how conductivity varies is a change in its value not a colour on an LED. Twenty-five years ago it was shown that is predictive ability requires a combination of the absolute quarter value, its variation with time and an inter-quarter comparison (3). The quarter sensors and

algorithms in AMS now allow this. The confidence levels on mastitis detection by farmers using AMS farms has soared. New results from New Zealand using in-line quarter sensors on a Merlin robot show a sensitivity of 92% and a specificity of 95% (5). This indicates that fine-tuning on the individual herd can make the system very practical

Colour sensors

There is nothing more normal than some blood in milk but not when the milk appears pink. AMS now come with milk colour sensors that not only detect when it is visibly pink but also can measure other shades indicative of other abnormalities or changes. Results as good as 100% sensitivity and >99% specificity are being reported for red blood cell counts lower than visibly detectable (13).

Next

The main AMS manufacturers and sensor companies are developing additional and sophisticated tests. They may be required more or less for AMS but commercial success is likely to depend on their applicability to all milking systems. A selection known to be under development follows. Some are still laboratory versions but several are in-parlour or cow-side prototypes or pre-marketing versions.

Projects have been reported on use of rheology and lactate assays but no data are available

ATP

Tests have been described over several decades determining ATP (adenosine triphosphate, the immediate energy store of all cells) as an indicator of the abundance of cells in milk from its ability to react with Luciferase and emit light. The major problem is to balance the cost of light detecting instruments with the amount of Luciferase required, an expensive reagent. Considerable attention appears to have been given to simplify and optimise the test as an indicator of cell count. The positive predictive value for cell count levels as low as 150,000 cells/ml appears impressive with sensitivity up of 95%. The sensitivity and accuracy of the test do not appear limiting just the potential cost.

NAGase

Real-time biosensors may offer the ultimate sensing opportunity. The electrochemical activity of the enzyme N-acetyl glucosaminidase (NAGase) may be an indicator of inflammation in the udder. An early report (6) showed its potential but no clinical data are available.

NIR

Near infrared spectroscopy (NIR) is used routinely to determine the composition of milk, fat and protein for payment. It can detect other, more specific changes in milk such as individual proteins.

Conventionally the equipment is very expensive but on-farm products for horticultural use are now available. Recent studies (10) suggest that these spectra may indicate accurately milk abnormalities within a few seconds. Spectra may be obtained from milk or udder tissue. They are highly detailed and require sophisticated algorithms for detection of changes. They may be ideal for quality control on AMS or as a veterinary tool

MAA

The inflammatory response in the udder is triggered by a number of chemical messengers that generally are too expensive to measure. Part of the response they induce is the production of acute phase proteins; several are well characterised, that further induce the immunological response of the animal. Amyloid A is easily detected very early (7) and a specific mammary gland version, milk amyloid A (MAA), is produced locally only. A commercial assay is available for this protein. It has the potential for a very early detection test and the sensitivity of the assay may allow detection of changes in bulk supplies.

DCC

In 2002 DeLaval launched on some markets, but not the UK, their Direct Cell Counter which determines cell count in less than one minute. There is a capital cost and a high recurrent cost for the single use cassette. The technology is getting closer to the cow but at an unsustainable price for farmer use.

SCC dipstick

A dipstick that purports to detect thresholds of milk cell count using the intensity of an antibody reaction has been described (1) from Canada. Its sensitivity appears similar to that of other cell count devices but data are not continuous. It appears to have a high predictive value for low cell count milk. Its limitations are on flow of milk in the device with high cell count milk and the 10 minutes reaction time considered too slow for a true cow-side test.

USING TESTS

We are hung up on what we think we know and that seeing is believing! This leads to a natural suspicion of anything new hence test evaluations tend to be compared to cell count. This may be a limitation to imagination,

of users but certainly not scientists. A change in cell count is only one parameter of the developing mastitis and not the earliest change.

We always want to relate indicators to SCC yet other changes are as indicative on their own – maybe we have to extrapolate the lesson when we converted from TBC to Bactoscan in describing milk hygienic quality? We could/should accept another, or additional, parameter(s). Several have similar accuracy, they are equally cheap and potentially much faster.

The tests described above all tell that milk is abnormal and give justification for excluding it from the saleable product. They give no real indication of what caused the problems and how to resolve it. The implication has always been that abnormal milk is caused by bacterial infection and that we are measuring the mastitis or response. A more specific test may give much more valuable information when the market is sophisticated enough to know that it wants more information. The choice of test may hinge on its accuracy but also on how early the information can be available and the timing of changes in milk shown below indicates the increased value of some tests.

Sequence of events in the development of mastitis.

Bacteria invade, multiply, metabolites produced, Time = 0-24h

      Signals released   - α-TNF, interleukins
                 - acute phase proteins *e.g.* MAA
                    Time = 8-24h

       Cell count (and ATP) rises   Time = 24-36h

             Epithelial damage
. . . .conductivity and NAGase rise..................
               Time = 36h

               Milk changes, volume and homogeneity
                  Time = >48h

Despite widespread scepticism on the value of milk electrical conductivity information, it now appears to be available from some systems in a form that the consumer appreciates. Colour sensing appears a valuable addition. Both these are in-line benefits from AMS that have a role in all milking systems.

More accurate data may be available from ATP and MAA tests and their potential is most likely in cow-side confirmatory tests e.g. after an alert from a conductivity sensor, or in milk quality control. NIR tests are much further from the market but offer much more potential in the information that can be gathered. This will be available quickly and involves a non-invasive

sensor; it does not go into the milk. On these bases they will be robust enough for all milking systems in ways that biosensors may never achieve. Their initial role is likely to be in AMS or in cow-side testing.

BACTERIAL IDENTIFICATION

The Gold Standard for mastitis investigations has been bacteriological examination of a milk sample, a process that takes in excess of 18 hours when the sample is in the lab, and often 48 hours, for several of the bacteria, to be confident of the results. Even then 15-25% of samples may give no indicative cause.

It we apply one of the primary screen tests described above we can get more confidence in the results from applying additional tests, AMS now do this, or wait for bacteriology. Cow-side bacterial identification is possible; dipstick prototypes are available. The components are being integrated into a multi-target test. It is clear that NIR spectral analysis may take testing much further as various characteristics of the spectrum can indicate the bacteria present and their precise effects on milk, possibly differentiating between infection and contamination.

REFERENCES

1.　Barratt, K., Leslie, K. and Bashiri, A. (2003) An evaluation of the Portascc™ test for determining udder health status in dairy cows at dry off. *Natn. Mast. Coun. Ann. Meet. Proc.* pp 280-281.
2.　Booth, J.M. (1988) Progress in controlling mastitis in England and Wales. V*et. Record* **122**: 299-302.
3.　Emanuelson, U., Olsson, T., Holmberg, O., Hageltorn, M., Mattila, T., Nelson, L. and Åström, G. (1987) Comparison of some screening test for detecting mastitis. *J. Dairy Sci.* **70**: 880-887.
4.　Malcolm, J.F., King, C.W. and Campbell, M.M. (1942) The value of cell content and electrical conductivity of milk as criteria of bovine mastitis. *Proc. Soc. Agri. Bact.* pp30-34.
5.　Mein, G.A., Sherlock, R.A. and Claycomb, R.W. (2004) Making sense of in-line sensing for milk conductivity. In: *Automatic milking.* Wageningen Per (in press).
6.　Mottram, T., Hart, J. and Pemberton, R. (2000) Biosensing techniques for detecting abnormal and contaminated milk. In: *Robotic milking*, eds H. Hogeveen and A. Meijering, Wageningen Pers.
7.　Pedersen, L.H., Aalbæk, B., Røntved, C.M., Ingvartsen, K.L., Sorensen, N.S., Heegaard, P.M.H. and Jensen, H.E. (2003) Early pathogenesis and inflammatory response in experimental bovine mastitis due to *Streptococcus uberis. J. Comp. Pathol.* **128**: 156-164.
8.　Schalm, O.W. and Noorlander, D.O. (1957) Experiments and observations leading to development of the California Mastitis Test. *J. Am. Vet. Med. Assoc.* **130**: 199-204.

9.	The Dairy Products (Hygiene) Regulations (1955) London: HMSO.

10.	Tsenkova, R., Morita, T., Shinzawa, H. and Hillerton, J.E. (2004) Detection of abnormal udder tissue and milk by near infra-red spectroscopy cow-side. In: *Automatic milking*. Wageningen Pers (in press).

11.	Whiteside, W.H. (1939) Observations on a new test for the presence of mastitis in milk. *Can Pub. Health J.* **30**: 44.

12.	Whyte, D.S., Orchard, R., Cross, P., Claycomb, R.W. and Mein, G.A. (2004) An on-line somatic cell count sensor. In: *Automatic milking*. Wageningen Per (in press).

13.	Whyte, D. S., Orchard, R., Cross, P., Wilson, A., Claycomb, R.W. and Mein, G.A. (2004) Seeing red: automated detection of blood in milk. In: *Automatic milking*. Wageningen Pers (in press).

WHEN I QUALIFIED MASTITIS WAS NOT A PAINFUL CONDITION

S. Borsberry
Veterinary Group, 608 Warwick Road, Solihull, West Midlands B91 1AA
Email: SBorsberry@aol.com

SUMMARY

Mastitis is a painful condition. A reduction in the number of farm staff and changes to milking routines may lead to bacterial infections and inflammation getting well established before the mastitis is recognised. Supportive treatment, including the use of analgesics, should be included in treatment protocols.

INTRODUCTION

The sight of a severe compound fracture of a distal limb or of an exopthalmia would result in a feeling of nausea to most observers. However, for most, empathy with a painful condition will depend on the person's previous experiences with his/her pain. No doubt trauma to teats may equate to finger injuries and an abscess on the buttock to a swollen inflamed udder. Welfare of animals has taken priority over the last decade with "cow comfort" becoming buzz words. Cow comfort would be more aptly described as life without discomfort.

Lameness in the dairy herd has been, and still is a serious welfare problem. In order to reduce lameness incidence and prevalence i.e. a reduction of a painful condition, it was and is promoted that lameness on farm has a deleterious affect of profitability, fertility, longevity and premature culling.

Having been asked to address the conference I attempted to ascertain whether farmers, herdspersons and veterinary surgeons perceived mastitis as a painful condition.

Attempting to devise a questionnaire for all "What does mastitis mean to you" (Table 1), I found that the question "Effect on cow e.g. discomfort/pain" had already implanted the suggestion of pain. Admittedly I could have rephrased the question but decided to abort the questionnaire.

Instead at routine fertility visits and meetings with other veterinary surgeons I engaged in non-lead discussions on mastitis.

At no time was discomfort or pain mentioned by any of the groups.

Table 1   Mastitis Questionnaire

(Please score: where 1 = very important and 5 = not important)

What does mastitits mean to you?	Score
Milk discard	
Treatment cost	
Effect on staff morale	
Interruption of normal milking routine	
Effect on cell counts	
Affect on cow e.g. discomfort/pain	

The results of this straw poll (Table 2) showed that the concerns were:

Table 2   Results of straw poll

Group	Concerns
Farmers	Economic effects of discarded milk and treatments
Herdspersons	Affect on normal milking routine
Veterinary surgeons	Time to treat toxic cows and problems associated with herds with elevated somatic cell counts

However, once the words discomfort or pain were mentioned it was obvious that they believed mastitis could be painful but was not at the forefront of their minds.

A survey, mainly of veterinary surgeons (6), showed there was considerable variation between individuals perception of the degree of pain suffered by mastitic cows. In my opinion the vast majority involved in the livestock industry are of the view that pain needs to be alleviated. Some veterinary surgeons are promoting (9) the administration of analgesics following routine procedures such as disbudding and castration. In no way is this an implied criticism of the veterinary profession or livestock industry. Attitudes have changed with the realisation that animals have rights and the education authorities placing greater emphasis on improved animal welfare which includes the prevention and alleviation of pain.   For most

practitioners assessing the degree of pain, particularly in mastitis, is subjective being based on

➢   General demeanour

➢   Response to palpation of a swollen quarter(s)

➢   Reluctance to walk

➢   Abducting hind limb away from affected quarter

There was an aspect of pain, which I was not aware of until I heard that cows with clinical mastitis exhibit allodynia i.e. have a decreased pain threshold and are hypersensitive to painful stimuli (5).

What causes pain in mastitis?

➢   Inflammation

➢   Increased intramammary pressure

➢   Increased external pressure e.g. from an adjacent limb

➢   Some observers believe toxic dehydrated cows may experience pain (4).

TREATMENTS

Approach

It has to be borne in mind that when pharmaceuticals are used outside data sheet recommendations, for example when:

➢   The number of intramammary tubes is increased

➢   The treatment period is increased

➢   The interdose interval is shortened

➢   The treatment is changed to another product

➢   Simultaneous administration of parentral antimicrobials

➢   Administration of non-steroidal anti-inflammatory drugs (NSAIDs) by routes not included on the data sheets

then the withdrawal periods must be not less than the standard 7 days for milk for 28 days for meat. There are further restrictions as many products are not licensed for food producing species including analgesics.

With the ever increasing pressure on the profitability of milk production and the decrease in manpower there is

> ➢ a reluctance to request veterinary visits
>
> ➢ an increase in clients seeking telephone advice
>
> ➢ reduced time available to attend to the needs of individual animals

Many veterinary surgeons supply pharmaceuticals for known health problems, which occur on a particular unit. However, it is essential that:

> ➢ treatment protocols are established
>
> ➢ they are only for use on that unit
>
> ➢ that there can be side effects e.g. when NSAID are administered above the stated doses and duration

Before describing the various treatments, which may be employed, it should be remembered that apart from acute toxic mastitis, which generally is readily recognised by farm staff, there are the infections and inflammations, which get well established before mastitis is diagnosed. Part of the problem appears to be:

> ➢ Less fore-milk examination
>
> ➢ Less pre-milking udder preparations
>
> ➢ Fewer experienced milkers
>
> ➢ Less time for individual cow care

Euthanasia

Although this appears drastic it needs to be considered with any case where the prognosis is hopeless *e.g.* gangrenous mastitis.

Therapeutic use of antibiotics

This subject is beyond the scope of this paper but the most appropriate choice of antibiotic will effect a bacterial cure and prevent further inflammation. However, their use to treat toxic coliform mastitis has been questioned (8).

Improved drainage

Overstocked udders

Although not a mastitis condition the logical method to alleviate pain is to relieve pressure by milking more frequently.

Blocked teat canals/viscous mastitic milk (not blind teats)

To aid a drainage problem, which can occur with summer mastitis or a traumatised teat, either amputation or longitudinal splitting of the teat may be beneficial.

Pre-partum physiological udder oedema

Again, this is not a mastitic condition but can lead to problems, it tends to be associated with peri-parturient heifers, (overstocked udders can occur in pluri-parturient cows), which can result in the conditions

> ➢ Necrotic dermatitis (udder seborrhoea)
> ➢ Excessive strain on the udder suspensory apparatus leading to a permanent pendulous udder.

Treatments may include

> ➢ Lubricant application between the udder and hind limbs to reduce friction
> ➢ Pre-calving milking
> ➢ Induction of parturition

Although every case must be assessed individually.

Oxytocin
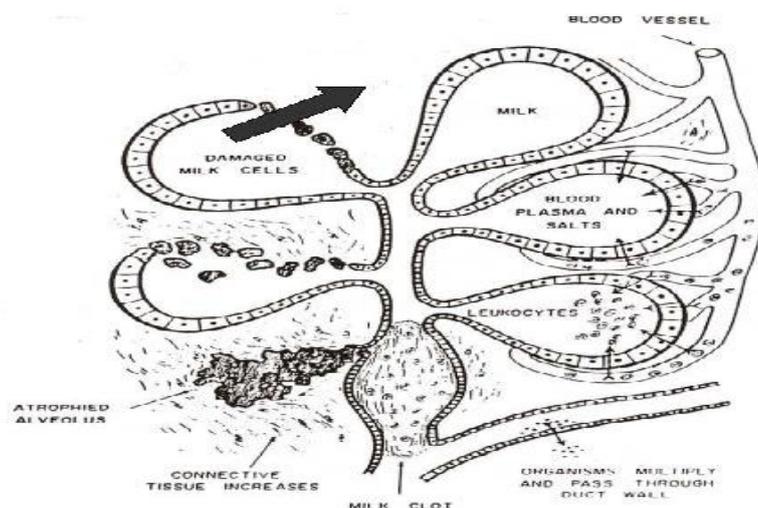
Oxytocin is frequently used to aid the stripping of affected quarter(s). However, I believe oxytocin is contra-indicated when

> ➢ The teat canal is obstructed
> ➢ In acutely inflamed udders especially when the cow is toxic. Many of these cows have obstructed milk ducts. Administration of oxytocin can increase the pain experienced by increasing the pressure within the udder and cause a greater flow of toxins etc. from the alveoli through the damaged alveolar walls and hence into the circulation (Figure 1).

Under these circumstances we may well be increasing the degree of pain.

> ➢ 100 IU i/m 12 hours apart - 3 treatments (11).
> ➢ 30 IU i/m approximately every 2 hours for 24 hours (8).
> ➢ 80 IU i/m followed by 20 IU 2 or 3 times daily (1).

Figure 1 The principal changes in mammary tissue during the development of mastitis (apologies to the author, source not known)

Calcium Borogluconate (Toxic cases)

Calcium borogluconate 40% 400 ml slowly i/v is advocated by some clinicians (8).

Fluid Therapy (Toxic cases)

Hypertonic Saline

7.5% sodium chloride solution - 2 litres i/v to encourage the cow to drink. However, the author has observed extremely variable results with this treatment. Perhaps a better system would be to deliver intra-ruminal fluids following i/v therapy.

Glucose Saline

Giving 10 litres i/v by gravity (3) or rapidly using a converted garden sprayer (2).

Isotonic Saline

45 litres (7) or 55 litres (10) given intravenously over a 24 hour period for severe dehydration.

Oral Fluids

The method preferred by the author is by apparatus specifically designed for intra-ruminal delivery - Selekt Cattle Pump - which has greatly facilitated the procedure. However, some authors suggest that where intestinal motility is reduced i/v fluid would be more beneficial.

25 litres isotonic saline (3)
50 litres "calf scour" type electrolyte (2)

Glucocorticoids

Glucocorticoids useful in the early stages of inflammation and of little value when dealing with established infections.

Intramammary preparations containing this type of corticosteroids are preferred by some staff as there is a rapid reduction in udder inflammation.

Parentral administration is not often used and the stage of pregnancy needs to be established.

Non-steroidal Anti-inflammatory drugs (NSAID)

Products include:

Ketoprofen	Ketofen 10% - Merial Animal Health Ltd
Flunixin	Binixin Injection 5% - Bayer plc Finadyne Solution - Schering-Plough Animal Health

These drugs have several properties
  ➢ Non-steroidal
  ➢ Non-narcotic analgesics
  ➢ Anti-inflammatory
  ➢ Anti-endotoxic
  ➢ Anti-pyretic

All of which are beneficial to the mastitic cow. These are extremely potent drugs and care must be taken not to exceed the dosage and duration of treatment.

Ketoprofen	2 g i/m daily for up to 5 days (12)
	3 mg/kg i/v or i/m daily for up to 3 days (author)
Flunixin	1000 mg i/v or i/m then 500 mg i/v or i/m every 12 hours for a maximum of 5 days

Clients report that where NASIDs are included in their mastitis treatment protocols

> ➤ It helps to reduce high temperature
>
> ➤ Dry matter intake recovers quickly
>
> ➤ Cow appears to feel better quicker
>
> ➤ Hardness of quarter rapidly reduces
>
> ➤ Staff are happier as cows respond quicker and there are less fatalities

CONCLUSIONS

There is an increasing awareness that mastitis is a painful condition. NSAID are primarily used for their anti-inflammatory properties but their analgesic qualities are underestimated. Rapid response to treatment and reduced fatalities when NSAID are included in treatment has a beneficial effect on staff morale.

REFERENCES

1.   Anon (2001) Compendium of Data Sheets for Veterinary Products (2002–2003) p 369.
2.   Blowey, R.W. (1993) The treatment of difficult cases of mastitis. *Proc. Brit. Mast. Conf.* pp 63-69.
3.   Faull, W.B., Hughes, J.W. and Ward, W.R. (1991*) A Mastitis Handbook for the Dairy Practitioner 5th Ed.* Liverpool University Press p 12.
4.   Fitzpatrick, J.L., Young, F.J., Eckersall, D., Logue, D.N., Knight, C.H. and Nolan, A. (1998) Recognising and controlling pain and inflammation in mastitis. *Proc. Brit. Mast. Conf.* pp 36–44.
5.   Fitzpatrick, J.L., Young, F.J., Eckersall, D., Logue, D.N., Knight, C.H. and Nolan, A. (1999) Mastitis - a painful problem? *Cattle Practice* **7**: 225–226.

6.   Fitzpatrick, J.L., Nolan, A.M., Scott, E.M., Harkins, L.S. and Barrett, D.C. (2002) Observers perceptions of pain in cattle. *Cattle Practice* **10**: 209–212.

7.   Green M.J. (1993) Treatment and prognosis of severe endotoxin - induced coliform mastitis: A field study. *1993 Cattle Practice 1:* 237-251.

8.   Green, M.J. (1995) Treatment and Prevention of Coliform Mastitis. *Proc. Brit. Mast. Conf.* pp 9–18.

9.   Lambert, C.A., Leonard, D.M. and May, T.W. (2003) Pain relief pays. www.lambertleonardmay.co.uk. Accessed 23rd May 2003.

10.   McGuirk, S.M. (2001) Developing fluid administration protocols in bovine practice. *Proc. 34th Ann. Convention Amer. Assoc. Bovine Practitioners* pp 6–9.

11.   Morin, D.E., Shanks, R.P. and McCoy, G.C. (1998) Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. *JAVMA* **213**: 676-684.

12.   Shpigel, N.Y., Chen, R., Winkler, M., Saran, A., Zig, G. and Longo, F. (1994) Anti-inflammatory ketoprofen in the treatment of field cases of bovine mastitis. *Res. Vet. Sci.* **56**: 62-68.

SCIENCE (OR ART) OF CELL COUNTING

Elizabeth Berry, Nicola Middleton, Michael Gravenor and J. Eric Hillerton
Institute for Animal Health, Compton, Newbury, Berks RG20 7NN

SUMMARY

A series of studies have confirmed press reports and commentary that there is significant variation both in bulk milk and individual cow cell counts. Several areas where variation can occur have been demonstrated. These include sample handling on farm and in the laboratory, and possible deviations from operating protocols suggesting poor attention to quality control. Additional wide variation both in bulk tank and individual cow cell counts within short time frames remain unexplained. The methods used to calculate the mean bulk milk cell count for payment reduces the potential error considerably. However, for individual cow cell counts the use of serial sample collected within a short period is advised.

INTRODUCTION

The somatic cells in milk are largely blood-derived leukocytes and the count or concentration is recognised as a measure of the infection status of the mammary gland and the quality of milk produced (6). Bulk milk cell counting in England and Wales was initiated in 1968 and carried out on a more regular basis from 1971 by the Milk Marketing Board. From 1977 this was increased to monthly for every herd and the data were supplied to the producing herds (2). Bulk milk cell counts were adopted as one indicator of milk quality and carried out by Central Testing Laboratories from 1990.

Following the termination in 1994 of the Milk Marketing Board and the Central Testing Laboratories, milk quality, including bulk milk cell counting, has been carried out by various laboratories under contract for the milk buyers. An inter-laboratory proficiency-testing scheme was set up in 1995 and is still running with participation being voluntary. There is also an international ring trial in operation with one Northern Ireland laboratory representing Great Britain. In a recent European Union trial organised by the Community Reference Laboratory 14 laboratories completed the trial. Only an Irish laboratory participated and reproducibility for all laboratories was reported as being poor.

In most developed dairy industries various regulatory limits are applied to milk used for human consumption. In the European Union the somatic cell limit is a maximum of 400,000 cells/ml in bulk milk (Dairy Products (Hygiene) Regulations 1995). The cell counts usually form part of a payment structure with discrete thresholds of concentration determining qualification for bonus payments or penalty charges. In the EU the regulatory level is

determined by a three-month rolling geometric mean. In the UK this is calculated from thirteen, approximately weekly, cell counts, from samples of bulk milk.

Individual cow cell counts are commonly used as a management tool for aiding in decision making and may be carried out by various laboratories. These may be on regular basis, such as the service provided by National Milk Records, or may be on a more ad hoc basis with samples taken and submitted by the farmer.

There are agreed international standards to which cell count testing laboratories should conform (8, 11) and how the results should be reported (9, 11). The European regulatory instruments require adherence to these standards and methods.

Recently, questions have been raised on the accuracy of some of the data produced by the testing laboratories in the UK. Andrews (1) complained of a poor level of agreement between testing laboratories and generally questioned the openness of the methods and control processes within the testing laboratories.

There are limitations to any system. It is known that individual cow cell counts will vary with conditions other than an intra mammary infection. Transient rises may be seen with oestrous, at times of stress or even with increasing lactation length and decreasing dilution of the normal cell concentration (3, 12). A rise in bulk milk cell counts is generally reported for herds in England and Wales in the late summer and this has been correlated with the predominant seasonal calving pattern. A seasonal rise is also reported by countries such as Ireland and is again attributed to a seasonal calving pattern.

Correct sampling routines are important and all milk buyers have a Standard Operating Procedure on how to sample a bulk tank correctly. Algorithms are used by companies such as National Milk Records to calculate cell count for cows and herds milking three or more times a day or when a pooled sample for the days milk is not available. Calibration systems are in place for the equipment used to carry out the cell counting with known samples inserted at regular intervals within a day's testing. Then there are various ring trials that are in operation ranging from inter-laboratory to large-scale international comparisons.

We report on a series of milk buyer cell count data, cell counts from farm bulk milk and individual cow cell count data that test the variability between measurements with time, and between laboratories. The intention is to show the variance occurring, any differences between laboratories and how these may arise. This should provide guidance on the reliability that may be placed on the cell count systems for evaluating bulk milk cell count quality and in aiding herd management.

METHODS

Several studies were carried out either on individual or bulk milk samples. In all cases our own sampling was carried out according to the relevant standard for bulk tank sampling (4). Samples were fixed and labeled according to the laboratory requirements. All laboratories used the fluorometric method to calculate the milk cell count, the Coulter counter method has been discontinued for milk quality testing. All studies were carried out either on the bulk tank or individual cows from the Institute for Animal Health. This herd is a Holstein herd.

Individual sample - Study 1

An 1800-ml milk sample from the IAH bulk tank was collected after morning milking then aliquoted into 54 sub samples of 15 ml and fixed according to testing laboratory requirements. These sub samples were distributed into 3 sets of 18 pots. A second sample from cows with a high cell count was aliquoted into three 15-ml samples and fixed accordingly. The second sample was interspersed by random selection of place in the sequence of bulk tank aliquots as sample number 17. These samples were sent to three laboratories that provide commercial cell count testing for milk buyers and dairy farmers.

Bulk tank - Study 2

Historical data on bulk milk quality and herd performance were obtained on the IAH herd. The milk quality data were the regular bulk milk somatic cell count measurements made as part of the milk payment system by the milk buyer and reported on the monthly statements. These measurements were made at approximately weekly intervals and reported both as individual results and as a three-month rolling geometric mean each month.

Bulk tank – Study 3

In a second bulk tank study, the bulk tank for the IAH was sampled, after the morning milking, daily for 28 consecutive days. This bulk tank contained the milk from approximately 220 cows. The fixed samples were stored at 4^oC and sent in batches to a commercial laboratory for routine determination of the milk cell count.

Individual cow - Study 4

Morning milk samples from 21 cows were collected for 33 consecutive days using the DeLaval Flomaster milk meters sampler. These cows were in mid lactation and had all been confirmed as pregnant. This study used milk from cows free of intra mammary infection by major pathogens at the start of the trial although three cows developed clinical mastitis in the 33 days of sampling. The cows have been allocated to three infection groups,

uninfected, persistently infected (coagulase negative bacteria or Corynebacterial infections) and clinical mastitis, for analyses.

These samples were aliquoted into 20-ml individual samples after thorough mixing and samples fixed according to laboratory requirements. One aliquot from each cow for each day was sent to each of laboratories A, B and C for milk cell count determination. During this study period 3 cows were treated for clinical episodes of mastitis.

Data analysis

Cell count data were entered in to Excel spreadsheets; an independent operator checked the accuracy of the entries. Statistical analyses were performed on log transformed data as recommended (9). Data from studies 1 and 3 were analysed using mixed models and the statistical software R (10). The factors 'laboratory' and 'infection status' were treated as a fixed effects, and cow and sample within cow (study 3) as random effects. In study 3, considerable heterogeneity in within-group variation was clear (highest due to clinical events), and therefore three separate models were fitted for uninfected, infected (not clinical) and clinical animals. Day to day variability showed no clear trend (study 3) and was modeled by assuming simple random variation about a mean. This assumption was checked using models that explicitly included time trends plus autoregressive error structures, but no improvement in the fit over the basic model was found.

RESULTS

Individual sample - Study 1

The cell count results obtained from the three laboratories are shown in Table 1. The first 16 sub-samples were obtained from the same supply. Laboratories A and C reported the same geometric mean (± SEM) of 182,000 (± 2,000) cells/ml whilst Laboratory B reported 187,000 (± 8,000) cells/ml. For these samples, there was no significant difference between labs and good agreement between samples (between sample (log) standard error = 0.06). There was no significant sample/lab interaction.

Sample 17 was from a separate source and intended to have a significantly higher cell count. The laboratories reported counts of 9,700,000 to 15,400,000 cells/ml, with no two results being similar. Samples 18 and 19 were from the same pool as samples 1-16. Laboratory A reported results within the mean ± one SEM of samples 1-16. The data from laboratories B and C appeared to have a carry-over effect, contamination of samples 18 and 19. Laboratory B reported sample 18 as 277,000 cells/ml (48% higher) and sample 19 at 208,000 cells/ml (still greater than the mean ± two SEM). Laboratory C reported sample 18 at 775,000 cells/ml (an error of 425%) with sample 19 at 215,000 cells/ml (still greater than the mean ± two SEM).

Table 1   **Cell count reported by all three laboratories for aliquots of the same sample**
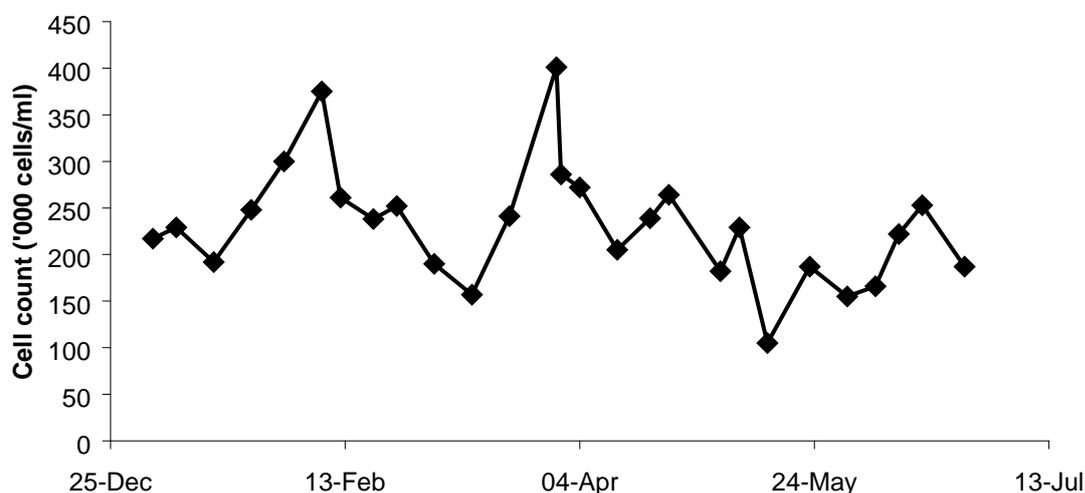
Sample	Lab A	Lab B	Lab C
1	192	195	228
2	191	191	203
3	181	182	170
4	186	198	172
5	192	184	177
6	190	182	172
7	186	180	184
8	179	195	181
9	177	192	171
10	173	200	211
11	180	180	186
12	178	183	176
13	179	181	176
14	172	185	166
15	179	177	185
16	177	187	169
17	15467	11495	9719
18	173	277	775
19	177	208	215
20	174	199	

Bulk tank - Study 2

The milk cell counts as reported by the buyer of milk from IAH over a 6 month period are shown in Figure 1. Over that period the arithmetic mean (± SEM) of 27 samples was 232,000 (± 12,000) cells/ml, the geometric mean was 223,000 (± 10,000) cells/ml and the range was 105,000 to 401,000 cells/ml. The results show wide fluctuations over the whole period and in shorter episodes (Figure 1). One example is the rise in weekly cell count from 157,000 cells/ml on 12 March to 401,000 cells/ml on 30 March and a subsequent large drop, to 280,000 cells/ml on 31 March. Other

fluctuations are smaller but commonly of 40,000-50,000 cells/ml from week to week.

Figure 1   Buyer's weekly cell counts for IAH bulk tank milk over a random period in 1990-1991

Bulk tank - Study 3

The daily cell count study used samples taken by trained staff to a proscribed procedure of agitating and hence mixing the tank properly, then sampling into a pre-labelled vial. Fixation and storage were also controlled. The testing laboratory reported cell counts that varied over a range of 84,000 to 282,000 cells/ml within 28 days but in more than one cycle of cell count fluctuations (Figure 2). The counts reported had an arithmetic mean (± SEM) of 178,000 (± 11,000) cells/ml and a geometric mean (± SEM) of 156,000 (± 17,000) cells/ml. The fluctuations were large, e.g. an increase in cell count between two consecutive days of 97,000 cells/ml, a doubling, when there was no obvious change in animal health; also the cell count twice dropped between days by approximately 80,000 cells/ml. Neither the tank volumes collected each day nor the farm records on events, treatments and medicines used showed no evidence of changes in cow number, production or mammary gland health suggest milk was withheld or added on any particular day.
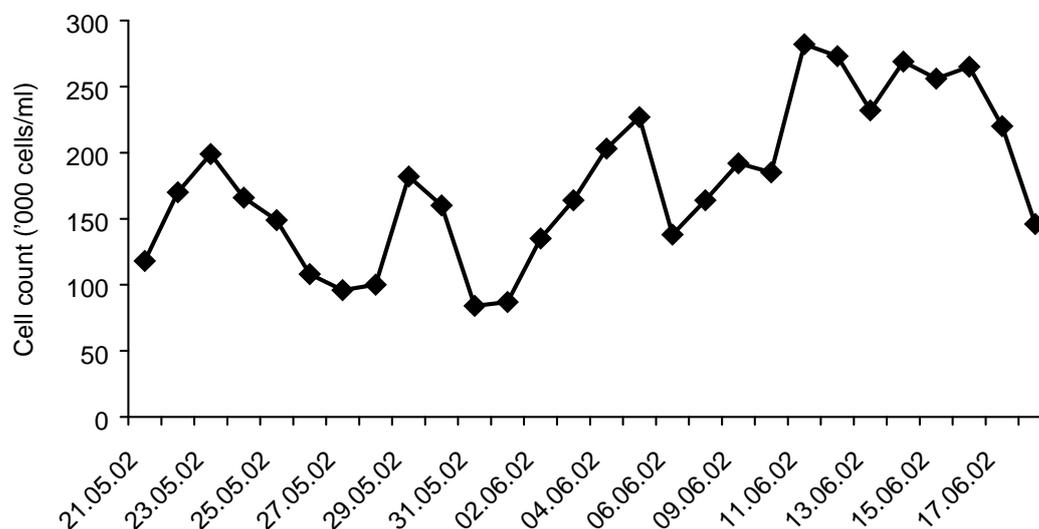
Individual cow - Study 4

Screening of data entered into the spreadsheet of results showed occasional data that appeared by observation to be unexpected from the data obtained on previous or following days.

Farm sampling errors could be attributed to 1% of the samples. These were identified by comparing results from previous and following days and

showed that in some cases the wrong cow had been sampled or sample bottles swapped between cows, both misidentifications. This was obviously equal for all laboratories.

Figure 2   Daily bulk tank cell count for IAH

Extreme inconsistencies between the three laboratories were discovered, i.e. the result from one laboratory differed markedly from those reported by the other two laboratories. A number of these individual events were identified for each laboratory. These appear to include sample misidentification in the laboratory or mis-reporting of sample number or a change of phase of sample vials in the counting process. In total, gross errors suggesting that the data were significantly wrong varied from 1.0 to 2.6% samples.

Removal of all data that appeared by observation to be suspiciously wrong produced a 'clean' data set. This data set was examined for the variation in cell counts between the three laboratories.   There were no statistically significant differences in the cell counts reported between the laboratories for the samples from infected and clinically diseased cows (Table 2). However, there were differences in the counts for uninfected cows.   The geometric means reported were laboratory A – 69,000 cells/ml; laboratory B – 55,700 cells/ml; laboratory C – 64,700 cells/ml. The trend for lower cell counts in laboratory B was found in 10/11 of the uninfected cows. The results did not differ between laboratories A and C (p=0.14) but were statistically significantly lower from laboratory B (p=0.001). The results do not tell which laboratory was correct.

Table 2   **Comparison of ln cell count data reported by three laboratories using a 'clean' data set**

Model	Fixed effect coefficients	
Uninfected cows	Integer	4.24
	Lab B	-0.22 (p=0.001)
	Lab C	0.07 (p=0.14)
Infected cows only	Integer	4.55
	Lab B	-0.005 (p=0.33)
	Lab C	-0.003 (p=0.96)
Clinically affected cows	Integer	6.1
	Lab B	-0.07 (p=0.72)
	Lab C	0.03 (p=0.88)

DISCUSSION

Errors in cell count data may occur from any of several events.

Misidentification and mishandling of samples

The cell count variations reported in Study 2 are unrealistic and suggest possible errors in the cell count data. Such errors could arise from sample mishandling at collection e.g. misidentification, incorrect procedure in obtaining samples, improper storage or transport, sample misidentification in the laboratory, an erroneous count from improper calibration, other machine fault or a carry-over effect from a previous high cell count sample etc. These results, reported from the milk buyer for IAH bulk milk, appears to confirm Andrews' (1) suspicions that variability in milk quality cell count data occur that cannot be explained by the dynamics of herd udder health. The results from the second bulk milk study on 28 consecutive days, eliminating potential errors in sample collection and sample misidentification, confirm these concerns and focus attention on the actual cell count results. Study 3 allowed examination of all the areas where errors could be introduced to cell counting. First, it showed that errors might be introduced on the farm, in this case by farm staff, at sample collection and identification. Some 1% of samples were observed to be wrongly attributed because of this type of error. Sample misidentification was also seen to occur for the third study both at the laboratory level.

Carry over

When three different laboratories were asked to count 16 replicates of the same sample (Study 1) they produced very similar results. However, in this study there is clear evidence that procedures in two of the laboratories are inadequate or not properly applied. The finding of a gross distortion in the cell count of the samples tested immediately after the high cell count sample suggests inadequate conduct of testing by laboratories B and C is possible. It is recognised by IDF (7) that a carry-over may occur and must be allowed for, especially in counts after a very high count. The carry-over must not exceed 2%. Laboratories B and C did not appear to allow properly for carry-over. Occurrences of this type of event would be of greater concern if the data were a series of individual cow cell counts. However, no bulk tank cell count in the European Union should ever approach 10,000,000 cells/ml because of the regulatory level and hopefully routine quality control should identify such an anomalous result.

Frame shifts and variation

Frame shift errors where a group of samples were out of phase often by one sample occurred for laboratory A. There was no apparent recognition of this misidentification and it may be that no mechanism exists to identify such a problem. Fewer and less obvious errors were attributable to the other two laboratories and in total 1–2.6% samples probably had the wrong cell count because of laboratory mishandling or identification. There were no apparent problems, false negative results, in identifying truly high cell count samples from both sub clinical and clinical mastitis. In study 4 the results for laboratories A and B were the more similar. This is in contrast to study 1 where results for laboratories A and C were similar.

When the consistency of data between the three laboratories is examined, i.e. when the result from one laboratory deviates from the results reported by the other two laboratories by more than 10%, then approximately 18% of results are outside this range. It must be stressed that this also occurs with the 'clean data set' too. The international standard (11) requires means to be within ± 5% at a cell count range of 100,000 to 200,00 cells/ml.

This means that for individual cow sampling to be worthwhile, it is very important to take several serial measurements over a short time period from individual cows if the cell count is to be truly representative of the individual cow mean. Repeated sampling is costly and not always practical. When using limited results for a cow a margin of error must be assumed of around 40,000 cells per ml.

CONCLUSIONS

Several areas for concern were highlighted from these studies ranging from possible incorrect sampling routine, misidentification of samples, laboratory

errors including unrecognised carry over effects, and normal variance. This highlights that the present recommendations on using cell counts are essential.

EU guidelines and IDF recommendations that results for bulk tank cell counts should be presented using the geometric mean calculation result in the effects of any extreme results being minimised. The method in the UK reduces the error on the 3-monthly mean bulk milk cell count to approximately 4%.

In uninfected cows any one cell count should be used with caution as a proportion of results reported have been found to be wrong. Serial testing on individual cows is probably essential in order to obtain a realistic representation of possible infection status of a cow. Management decisions should not be made on one cell count result and variability in cell counts must also be taken into account. A margin of error, perhaps up to 20%, should be assumed when using limited results.

This study demonstrated that individual cow cell counts, even in uninfected cows, vary from day to day and that this can be more than 40%. Reasons for this variance are still not fully understood and need to be explored more.

Cell counting has been used over the last 30 years to give an indication of milk quality primarily for bulk milk and this was extended for use in individual cows. When used correctly it gives an estimation of bulk milk quality and potential infection status of the individual cow. However, there are limitations as highlighted and its use may eventually be replaced by more recent technology.

REFERENCES

1.   Andrews, A. (2002) Mastitis and somatic cell counts. Letter. *Vet. Rec.,* December 7, pp 711.
2.   Booth, J. (1988) Progress in controlling mastitis in England and Wales. *Vet. Rec.* **122**: 299-302.
3.   Brolund, L. (1985) Cell counts in bovine milk. Causes of variation and applicability for diagnosis of sub-clinical mastitis. *Acta Vet. Scand. Suppl.* **80** - thesis.
4.   BS ISO (1997) Milk and milk products. Guidance on sampling. BS ISO 707, British Standards Institute, London.
5.   Dairy Products (Hygiene) Regulations (1995) Statutory Instrument No. 1086, HMSO, London.
6.   Dohoo, I.R. and Meek, A.H. (1982) Somatic cell counts in milk. *Can. Vet. J.* **23**: 119-125.
7.   IDF (1981) Laboratory methods for use in mastitis work. *Bulletin of the International Dairy Federation* No. 132, IDF, Brussels, pp 27.
8.   IDF (1995) *International Dairy Federation Standard 148A, Enumeration of somatic cells.* IDF, Brussels, pp 8.

9.   IDF (1997) Recommendations for presentation of mastitis-related data. *Bulletin of the International Dairy Federation* No. 321, 6-25, IDF, Brussels.

10.   Ihaka, R. and Gentleman, R. (1996) R: A language for data analysis and graphics. *J. Comput. Graph. Stats.* **5**: 299-314.

11.   ISO (1997) *International Standard, Milk – Enumeration of somatic cells – Part 3:* Fluoro-opto-electronic method. ISO13366-. International Standards Organisation, Geneva.

12.   Laevens, H., Deluyker, D., Schukken, Y.H., De Meulemeester, L., Vandermeersch, R., De Muelenaere, E. and De Kruif, A. (1997) Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows. *J. Dairy Sci.* **80:** 3219-3226.

WHAT CAN I GET THE MILKER TO DO?

Ian Ohnstad

ADG, New Agriculture House, Blackbrook Park Avenue, Taunton TA1 2PX
Email: ian.ohnstad@adas.co.uk

SUMMARY

The modern milker in 2003 is under increasing pressure. This usually means milking and tending more cows than ever before, in an attempt to be profitable.

Considering the work routine during milking allows realistic performance figures to be determined but also highlights weaknesses in the existing system.

Introducing a consistent teat preparation routine will not only improve hygienic milk quality but can improve parlour performance, boost yield and improve teat end condition.

Thorough teat disinfection after milking with the correct product not only helps control mastitis but can also maintain or improve teat skin condition.

INTRODUCTION

In response to the question, 'What can I get the milker to do?', it is easy to make the glib response ' less and less!'.

The UK dairy industry is going through a period of rapid re-structuring with registered milk producing holdings falling consistently. At the same time, national herd size is falling, albeit at a lower rate.

This is demonstrated in Table 1 using data from the annual DEFRA census. This data relates to the situation in England.

Table 1   Number of holdings and dairy cows in England

Year	No. of holdings	No. of dairy cows
1990	22, 793	1,997,685
1995	19, 632	1,809, 282
2000	15,219	1,575,320
2001	14, 106	1,490,226
2002	14, 342	1,462,155

Because the size of the dairy herd is falling more slowly than the number of registered holdings, average herd size is increasing.

The percentage of herds with more than 100 cows is also increasing. This is demonstrated in Table 2.

Table 2   Average herd size and % herds > 100 cows in England

Year	Average herd size	% herds >100 cows
1990	87	n/a
1995	92	45
2000	103	50
2001	106	53
2002	107	56

Seldom is an increase in herd size associated with an increase in available labour. This invariably results in an increased workload and pressure on staff.

DISCUSSION

When an advisor or consultant is invited to visit a dairy farm, it is invariably in response to a problem. The somatic cell counts (SCC) may be increasing, the rate of clinical mastitis is excessive or Bactoscans (BS) may be unacceptably high.

Almost all problems are multi-factorial and it is essential to identify actions or changes in routines that are required by the milker and those factors, which relate more closely to the farm owner or manager. It is important all parties are involved in the discussions and are aware of the 'bigger' picture, although this has to be handled sensitively as it can sometimes lead to a reluctance to shoulder individual responsibility.

When undertaking an investigation, it is important to consider the pressure the milker may be under. The recommendations should be prioritised and in many cases the concept needs to be sold to the individual. While highly commendable to discuss the theoretical background to a recommendation, in many cases all the milker will want to know is how much extra work this involves and what immediate benefits will be noted.

When attempting to change the behaviour of a milker to improve milk quality, there will inevitably be a number of points to consider. However, within the scope of this paper, I have focussed my attention on the milking routine.

A   Recognise the limitations of the milking routine

As pressure to improve parlour performance in term of cows milked or litres produced per hour increases, a good milking (work) routine is often the first casualty.

Table 3 demonstrates the effect of different work routines on parlour performance (2)

Table 3   Effect of work routine on parlour performance

Work routine	Routine 1 (sec/cow)	Routine 2 (sec/cow)
Let in and feed	10	5
Foremilk	6	-
Pre-spray	6	-
Prepare teats	12	-
Attach cluster	10	10
Remove cluster	-	6
Post spray	3	3
Let cows out	10	5
Total	48	29
Max cows / hr	75	125

The milking routine, rather than the number of available milking units, is usually the factor which limits parlour performance. Examining and timing the routine employed on farm can often prove useful. This can help identify areas where the routine can be improved and time saved without compromising milk quality. If time can be saved on parlour loading, cow feeding, parlour unloading or animal separation this should reduce the need to cut corners in teat preparation or teat disinfection. These data are optimal times, often they may be greatly exceeded or highly variable between cows or batches of cows.

B   Recognise the importance of teat preparation

The role of teat preparation in milk hygiene is well understood. The role of teat preparation in stimulation and milk ejection is often understated. While most milkers would view 20 seconds of pre-milking teat preparation as onerous, particularly if milk quality is satisfactory, it may be adopted if the milker believes it will improve milking performance.

Improved duration and thoroughness of teat preparation will usually improve milk quality.

Table 4 summarises the effect of teat preparation method on milk quality (3).

Table 4   Effect of teat preparation method on milk bacterial content

Method	Experiment 1 (cfu/ml)	Experiment 2 (cfu/ml)
No udder prep	6,380	11,091
Dry paper towel	6,117	8,021
Teats wash and dry	6,130	6,314
Disinfectant wipe (single use)	4,695	3,555
Pre-spray	2,938	2,976

This improvement in teat cleaning is likely to manifest itself in an improvement in milk quality. To capture the full benefit of teat preparation, the activity has to be carried out for around 20 seconds/cow. When milking a 500 cow herd, this requires 2.5 hours per milking.

However, spending more time on teat preparation will also improve the milk let-down stimulus and therefore milking installation performance. The 'ideal' time spent preparing teats to maximise oxytocin release will vary depending on stage of lactation. As a rule of thumb, 20 seconds/cow, is considered a good compromise across different stages of lactation.

If it can be shown that the 20 seconds/cow is actually an investment of time and the return in terms of reduced unit on time is considerably greater than the time invested, then the practice is more likely to be adopted.

However, for this principle to be successful, there are a number of fundamental actions that have to be understood and implemented.

The milk let-down reflex is not immediate. In response to a consistent stimulus, oxytocin is released from the pituitary gland in the brain into the blood supply. When the oxytocin reaches the udder it stimulates contraction of the myoepithelial cells which surround the milk secreting alveoli.

Manual stimulation of the teats activates sensory nerves causing relaxation of the smooth muscle around the milk lobes, cistern and in the teats. This process allows the cisternal volume to enlarge as milk ejection occurs and alveolar milk flows to the teat and udder cistern (1).

The amount of cisternal milk increases with the time between milkings. The amount of cisternal milk is relatively low during the first four hours after milking but then increases rapidly. With a milking interval of around eight

hours, a late lactation cow will yield around 1.5 kg of cisternal milk while a fresh calved animal will yield around 3.5 kg of cisternal milk.

If the timing of unit attachment does not coincide with oxytocin release, the movement of milk from the alveolar storage to the cistern will be delayed. There will be a temporary cessation in milk flow, when the cisternal milk is removed, before the appearance of the alveolar milk. This is termed bimodal let-down. Bimodal let-down can be seen as either a partial or complete stop in milk flow. It is often seen as a dry clawpiece shortly after unit attachment.

It has been suggested that large volumes of cisternal milk, associated with cows in early lactation, may limit the complete ejection of alveolar milk (5). Therefore, it is important that some of the cisternal milk is removed before milk let-down actually occurs.

The period between teat preparation and unit attachment is critical. This period is commonly referred to as prep lag time. Prep lag time is the time between beginning of teat preparation to application of the milking machine.

Studies in USA and Denmark have demonstrated that prep lag time is the most important factor in optimising milk efficiency (8). The optimal prep lag time will vary depending on stage of lactation. However, it is generally accepted that between 60–90 seconds is optimal across all stages of lactation.

Standard prep lag times throughout a lactation can increase lactation milk yields by 5.5% compared with a milking routine with a variable prep lag interval (7). This equates in a year to an extra 500 litres from a 9000 litre cow.

In addition to an increase in milk yield, if both the amount of preparation and the prep lag time are optimised, unit on time and the low flow/high vacuum periods can be reduced. It is said in the USA that on a typical dairy cow, an investment of 20 seconds in teat preparation will reduce average unit on time by 90 seconds.

Where these circumstances have been achieved, the ACR detachment points should be adjusted removing the unit earlier at the end of the milking. It is now common practise for herds milked twice daily to have ACR switch points set at 400 g/min with a five second delay. Herds milked three times daily have switch points set at 700 g/min and a five second delay. This compares with the default switch point of 200 g/min and a 25 second delay.

An additional benefit from ACR adjustment is a measurable reduction in teat end hyperkeratosis. This is thought to be related to the reduced time that the unit is attached to the cow when milk flow rate is <1.0 kg/min. Consultancy work in the UK, on farms with a hyperkeratosis problem, has demonstrated an average improvement in teat end hyperkeratosis of 0.9 points (Scored on a 1–5 scale) after adjustment of ACR. Although it has

proved hard historically to form an association between hyperkeratosis and SCC or new mastitis infections, a paper from Holland has recently provided an alternative perspective (6).

It must be stressed that ACR should only be adjusted if there is total confidence that a new routine has been adopted which includes at least 20 seconds of teat preparation and a prep lag time between 60 and 90 seconds.

C   Recognise the importance of teat disinfection

The importance of teat disinfection post-milking was highlighted in the NIRD Five point Mastitis Control Plan in the 1970s. It has proved to be particularly useful in the battle against contagious mastitis infection.

In addition to the role as a disinfectant, teat sprays or dips have a critical role in re-instating or maintaining teat skin condition. A well-conditioned teat with soft supple skin is better placed to withstand the rigours of machine milking.

There are a multitude of teat disinfectant products available in the market place today. When choosing the product it is important to consider whether the product is a concentrate or ready to use, the pH of the product, the level of skin conditioners (emollients) and the composition of the emollients. This was fully described at this conference in 2002 (4).

It is also essential that the method of delivery is considered. On average, dipping will give more uniform teat coverage than spraying. To optimise teat condition, it is essential to understand the combined role of teat disinfection, to disinfect and condition.

CONCLUSIONS

The milker in 2003 is under increasing pressure having to milk and tend more cows than ever before in an attempt to remain profitable.

Considering the work routine during milking allows realistic performance figures to be agreed, but also highlights weaknesses in the existing system that may not be related to the work routine.

Introducing a consistent teat preparation routine will not only improve hygienic milk quality, but can improve parlour performance, boost yields and improve teat end condition.

Thorough teat disinfection post milking with the correct product not only helps control new mastitis infection but can also maintain or improve teat skin condition.

REFERENCES

1.   Bruckmaier, R.M. (1998) Oxytocin release and milk removal in ruminants. *J. Dairy Sci.* **81**: 939-949.
2.   Dodd, F.H. and Griffin, T.K.   (1978) Milking Routines, Machine Milking, *NIRD Tech Bulletin* 1.
3.   Galton, D.M. (1984) Effect of teat preparation on bacterial populations. *J. Dairy Sci.* **67**: 2580–2589.
4.   Hemling, T. (2002) Teat condition – prevention and cure through teat dips. *Proc. Brit. Mast. Conf.* pp 1-14.
5.   Knight, C.H (1994) Milk accumulation and distribution in the bovine udder during the interval between milkings. *J. Dairy Res.* **61**:167-177.
6.   Neijenhuis, F (2001) Relationship between teat end callosity and occurrence of clinical mastitis. *J. Dairy Sci.* **84**: 2664–2672.
7.   Rasmussen, M.D. (1990) Comparison of a standardised and variable milking routine. *J. Dairy Sci.* **73**: 3472–3480.
8.   Rasmussen, M.D. (2000) A review of milking preparation: the science. *Proc. 39th NMC Ann. Meeting,* Atlanta, Georgia, pp 104–110.

RAPID MILKING. QUALITY MILK – WHILST MAKING A PROFIT

David Christensen
Kingston Hill Farm, Kingston Bagpuize, Oxon. OX13 5HY
Email: christensen@clara.co.uk

SUMMARY

In this paper the author describes how the milking of 400 cows is carried out on a farm in the south of the UK. A new parlour installation is briefly described but the paper concentrates on the everyday details of how the herd is milked quickly and the steps taken to endeavour to produce milk of high hygienic quality.

BACKGROUND

Kingston Hill Farm is a tenanted farm extending to some 840 acres located in the Thames Valley, 10 miles to the west of Oxford. It has been run by Christensen partners since 1968 and now has a milking herd of 460 Friesian cows with all herd replacements and all male calves also reared on the farm. The herd calves all the year round and averages 8,500 litres of milk sold per cow per year with rolling concentrations of butterfat at 4.22% and protein at 3.52% This year we should sell some 4 million litres of milk to the co-operative Milklink.

Some 5 years ago we were faced with what I hope will be a once in a lifetime decision. Our existing milking facility, a rather dilapidated 24-point herringbone parlour, had seen better days and milking was taking an increasing amount of time. At that stage we were milking for some 9 hours a day and this was with a lower level of daily milk production compared to today. We had a team of high quality staff who we were very keen to hang on to and, with our desire to increase our level of milk production, it became apparent that it was time to get stuck in properly for the long term or get out.

We would never claim to be the worlds best or lowest cost milk producer but like to think that we are reasonable operators and can produce a sufficient profit each year to pay the rent, reinvest in the business, make a living and finally keep Her Majesty's Treasury out of trouble. Given my personal shortcomings in diplomacy, patience and general tact, diversification and getting 'much closer to the customer' did not appear to be a terribly good idea, so it quickly became apparent that wholesale milk production was our future. Now we needed to find a means of milking large numbers of cows that fitted in with our plans.

THE SOLUTION

Swing-over herringbones, rapid-exit herringbones, inside milking rotaries, external rotaries and robots name but a few of the options that loomed up in front of us. We had a gut feeling that it was a large rotary that we needed but felt it was important to examine all the options and so enlisted the help of Ian Ohnstad of ADAS. What were we trying to achieve?

➢ Short overall milking times – with the potential for herd expansion. Ideally a working day that started at 5.30 a.m. and finished at 5.30 p.m. with some time off in the day as well. Finding and keeping good quality staff will be our biggest challenge in the future.

➢ Quality milk production – our customers expect it and to maximise our milk income. It goes without saying that rapid milking is irrelevant if poor milk quality is the result.

➢ A simple parlour – for a lower capital cost, lower running costs and for reliability in operation. We have never been huge fans of electronics anywhere on the farm but in the parlour they seem to us to be a long-term cost rather than benefit.

To cut a long, decision-making process short we decided that a large outside milker rotary would satisfy all the criteria and so the next step was to identify suitable manufacturers and see what they had to offer. After a lot more research and work Fullwood Ltd were selected to supply and install a 60-cow outside milking rotary parlour. The parlour started off as a 40-cow rotary but both my father and grandfather told me to stop playing at it and make sure I built it big enough, on the basis that everything they had ever done had needed expanding at a later date. I am glossing over the amount of time spent on getting the design, layout and cow flow correct in the new parlour because time doesn't permit today, but suffice to say that time spent at this stage can make a huge difference later on.

THE RESULT

In February 2001, on the day that Foot-and-Mouth Disease broke out in the UK, we started milking in the new parlour. We thought it was important that we tried to minimise the change-over stress on both the cows and staff and so for two days prior to the first rotary milking we gave the cows a dry run on the platform. As the cows came out of milking in the old parlour they were put on the rotary and given a rotation to start to acclimatise them to the process. When I say put, I really mean push and shove as we practically shoulder-barged all 330 cows on to the platform. After repeating this for a second day, again on the third day the first milking loomed. The last installation job was to move our existing bulk tank over which meant at that stage there was no going back – although I weakened somewhat here and bought 200 metres of alkathene pipe so if we got into a real pickle we

could at least milk through the old parlour and pump the milk over to the tank. Thankfully this was never needed and the first milking went extremely well taking the same amount of time as the previous milking in the old parlour. From there we have never looked back.

MILKING TODAY

Currently we are milking around 400 cows on a daily basis and the parlour operates with two men. The first man is the cups-on operator who is responsible for pre-milking hygiene and putting the cups on. During the grazing season, when all the cows are out grazing both day and night, we are usually presented with very clean cows and clean teats so units are attached without any teat preparation. A network of concrete roads and reasonable quality gateways helps facilitate cow cleanliness at grazing. However, if it is a wet day and the udders are wet or dirty we wipe with a medicated wet-wipe – in our case currently a Teisen medicated paper towel. A separate towel is used per cow and then discarded. In the winter when all cows are housed then every cow will be wiped with a medicated towel.

Winter housing is all cubicle-based with sand as the bedding material. All quarantined cows, both fresh calvers and mastitic cows, are milked last of all for two reasons. Firstly, to reduce the risk of cross contamination of mastitis pathogens between cows and secondly to allow sufficient time to treat them properly. You always feel under pressure when the platform is stopped when you are milking the main herd and that pressure will inevitably lead to short cuts being taken. Milking them last also minimises the risk of putting milk from a cow treated with antibiotic in the bulk tank. Both milkers in the parlour have a bucket of Sorgene solution nearby to disinfect units that have been in contact with mastitic cows and units are always dipped before being attached to fresh calvers.

Two other actions help to keep cows clean and these are keeping tails well clipped and udder flaming. Whenever a cow enters the parlour for the first time after each calving we will clip her tail and the area over her freeze brand and we have a battery-operated clipper to facilitate this task. When the whole herd is getting a bit unkempt we will spend a milking with another man in the parlour clipping all the cows tails and for this we have a mains operated clipper. To remove unwanted hair on the udder we use an udder singe device, suggested to us by Ian Ohnstad who, after suggesting the idea, issued a disclaimer requesting that we wait until he was next out of the country and the fireman's strike had ended before we tried this device. If the whole herd went up in flames he didn't want to be within lynching distance until I had cooled down a bit! However, he needn't have worried, as the device is very easy to use, very cheap to run and very effective. The wearing of a good set of waterproofs is to be recommended as the cows do take some level of exception to the smell of burning hair and react in that time-honoured fashion that cows do so well! Around once every 6 weeks, or as required, the udder hair is singed off the cows. Finally, good fly control is

a very important factor to stress-free milking and so all cows and young stock are treated with 'Spot-on' throughout the summer and early autumn.

The second operator in the parlour is of course the cups-off operator. The most commonly asked question from visitors to the parlour is why we didn't install ACR and an automatic teat sprayer and do without the cups-off operator? The short answer is that when we were doing our research the rotaries with this sort of set-up were not producing high quality milk. Nobody was checking those cows udders after the milking unit had been removed so all mastitis checking was expected to be done by the cups-on operator who simply hasn't time to do this properly and achieve a suitable output from the parlour. We have the high flow rate 'Ambic' in-line mastitis detector, which the cups-off operator uses as a tool to check for the evidence of clots post-milking.

The automatic teat sprayers that we saw working at this time were highly effective at covering every part of the cow in teat dip other than the udder and so this of course was a major concern to us and, without installing automatic identification and drafting, we needed an operator at the cups-off position to draft out cows. The technology will have improved since then and at some stage we must revisit and review this area but for now we are very happy with the system of two men milking and a very simple direct to line parlour with minimal electronics.

Getting cow flow right is absolutely critical if these sorts of parlours are going to achieve high throughputs. We were very fortunate to have as a close family friend John Gerring of Waikato UK who is an expert in this field and so we took on board all of his advice. A well-lit, clutter-free approach to the parlour with minimal corners and no sharp turns, with the same philosophy required for the exit. The whole site is very well earth bonded to allow any stray voltage to earth with minimal effect on the cows and cow drafting is performed by a simple rope operated gate with minimal diversion for the cows. Twelve front-opening stalls are very valuable for any routine cattle work like serving or pregnancy scanning. Our cows almost fight to get on the parlour platform – and that without starving them, although when on full winter rations they quickly become rather lazy and the use of a backing gate is invaluable.

Operator comfort is very important and a few simple ideas can make the world of difference. The cups-on operator stands on a thin plastic mat, which helps keep his feet warm in winter and helps prevent backache all year round. A large fan is positioned behind him for those very warm summer days, although we don't use it that often because the parlour is very well ventilated anyway. Having everything to hand minimises downtime and a good water system facilitates keeping the parlour clean during milking. For the cups-off operator again it is a case of trying to facilitate the whole process and so teat spraying points are located at suitable intervals for the last half of the parlour. Suitably positioned plastic shelves hold the important tools like the California milk test kit and the battery-operated

clippers.   The two men swap roles at each milking.   The key factor in keeping operators happy, however, is short milkings and a sensible length of working day.

Milk recording is done on a 6-weekly basis by ourselves; primarily as a tool for cell count management. We have a set of Waikato milk meters which we install in the parlour for two milkings, but we only sample from one milking. I deliver the samples to the On-Merit laboratory, which is some 35 minutes from the farm, by 9 a.m. and the cell count results are back on my fax machine within 4 hours so we can be taking any necessary corrective action during the next milking. This action might include checking cows that have a high cell count, but show no clinical signs of mastitis with the California milk test and then treating affected quarters. It might mean drying off cows earlier than planned, or simply that if a cow is due to be culled then the time has arrived for her to go. On-Merit provide a valuable low cost service which is very useful in keeping cell counts under control.

A brief word about drying off, if we have a number of cows to dry off then we don't try and do it during milking. The cows to be treated are separated and then we dry them off on the platform, but after milking has finished. Trying to dry off properly during milking when under pressure is a recipe for problems. All cows are treated with Orbeseal teat sealant. For those cows with higher cell counts, or that have had mastitis, they are treated with Cepravin dry cow tubes and Orbeseal.

CONCLUSION

So does all this rhetoric work? The parlour has been in operation for over 2½ years now and some 8 million litres have been collected. It has been very reliable and can milk cows at a tremendous speed. At the grazing time of the year, when no, or minimal, teat preparation is required, it takes a little over 2 hours from walking in to going home to milk some 400 cows. In the winter, when all cows are being wiped, this time will increase to 2½ hours. We also have the capacity to milk many more cows without adding to the overall milking time, because for every 60 extra cows we are only looking at adding the time of one rotation which is some 10–12 minutes (my father and grandfather were right!).

The bulk tank Bactoscans run at around 15–30,000 impulses/s in the summer and 20–35 during the winter. Cell counts run at around the 120,000 cells/ml level during the winter but nowadays increase to around 190 during the summer months, which we can only suggest may be due to heat stress and the fact that the cows have to work harder for a living. The number of cases of mastitis levels is coming down to below the national average of 35 cases per 100 cows and there is plenty more scope for ironing out weaknesses here. Our lack of fore-milking is an issue for our dairy hygiene inspector and we are on yearly inspections as a result, which I struggle to reconcile with the quality of milk that we are producing. As you

may imagine I have to summon up every last ounce of that minimal tact that I possess when the inspector arrives but we always agree to disagree amicably. But on the whole we like to think that we are achieving the title of this paper – 'Rapid Milking, Quality Milk – whilst making a profit' and are very confident that we can continue to do so.

CELL COUNT ANALYSIS USING InterHerd

Sarah Richards, James Hanks and Andrew James
VEERU, School of Agriculture, Policy and Development, P.O. Box 237, Reading RG6 6AR

INTRODUCTION

The analysis of milk somatic cell counts (SCC) at an individual cow level has recently become an even more important part of mastitis control than in the past. Reasons for this are:

➢ financial bonus schemes from dairy companies;

➢ new, non-antibiotic treatments for mastitis, requiring good understanding of SCC profiles in the previous lactation; and

➢ the requirement to secure profits in the narrowing profit margin environment of dairy production

Cell counts are not easy to analyse on a paper-based system. Computer-based herd management software can be used to sort and present the data in different ways that add value to the information. Conclusions on individual cows can be drawn from a variety of different data analysis routes. This type of analysis offers a veterinarian new opportunities to provide new services to existing clients and to recruit new clients.

SCC ANALYSIS WITH InterHerd™

Whilst there are various herd management programs that can analyse SCC data from various sources, InterHerd™, developed by VEERU and Pan Livestock Services as a replacement for DAISY, offers the most flexible analytical system currently in the market.

This project has developed various analytical models for monthly individual cow SCC recording data and implemented these models as part of routine, graphic outputs from InterHerd™.

This poster demonstrates:

➢ The financial losses that are incurred if low cell counts are not achieved

➢ Analysis of latest milk recording data

➢ Somatic cell count trends at herd level

➢ Analysis of individual animal cell counts

➢ Mastitis incidence over time

➢ SCC in the latest 2 lactations

CELL COUNT TRENDS IN UK RECORDED DAIRY HERDS

C. Smith and H. Richardson

National Milk Records plc (NMR), Fox Talbot House, Greenways Business Park, Bellinger Close, Chippenham, Wilts SN15 1BN

SUMMARY

Herd information and cell count data collected from NMR recorded herds have been analysed. The results show that there are clear rising trends in cell count associated with stage of lactation, lactation number and herd size. Cell counts are not largely influenced by geographical area, and most herds safely fall into the 151 to 200,000 cells/ml bracket. Individual animal cell count testing identifies where only a few cows contribute unduly to the herd average, suggesting that these results are just as important as bulk milk testing.

INTRODUCTION

The NMR database holds records for 9.5 million animals and over 32 million lactations. Individual milk samples have been tested for somatic cell count (SCC) since 1985. NMR currently collects records from more than 8000 dairy farms spread all over the UK. The majority of these farms are recorded monthly, with 3, 6, 8 weekly and quarterly frequencies also chosen by other herds. In a typical year, over 730,000 fresh lactations are completed and some 8 million individual samples tested for SCC. Other information relating to both individual cows and their herds is recorded, including herd size, location and reproductive performance. This data can then be used together with the cell count information to give insights into trends across the whole of the UK.

MATERIALS & METHODS

Milk samples are collected and tested at a single laboratory using 'Combi Foss' apparatus. These utilise a DNA staining technique to detect somatic cell counts, using an optically active dye (Ethydium Bromide), followed by counting electro-fluorescent reflections under UV light. Data are stored in a central database, from where it can be collated to provide simple statistics on SCC trends.

RESULTS

Cell count by herd size

The average herd size for NMR herds is currently 126 cows, with just under half the herds falling into the 101 to 200 cow herd size. The herd size band with the highest cell count is the 301+ cows.

Cell count by lactation number and by stage of lactation

The results over time show that older cows have higher cell counts. This is consistent across all lactation numbers since 2001. Late lactation cows also have higher cell counts, this applies to a mean average cell count as well as the median value.

Cell count by DEFRA region

Cell counts do not vary widely by region in Great Britain, apart from the Channel Island herds (Jersey and Guernsey) whose cell counts are 20% higher than the other regions. However, all regions have displayed a consistent upward trend over the last 5 years.

Percentage of herds and animals in each cell count band

The highest percentage of herds fall into the cell count band 151 to 200,000 cells/ml. For individual animal results, the highest percentage of animals have fewer than 50,000 cells/ml.

DISCUSSION

It is expected that cell counts will be highest in larger herds, due to more cows per man and therefore less time available per cow to manage cell counts. Overall cell count rises as herd size increases but this does not affect the majority of herds. Cell counts are generally higher in late lactation when milk yield drops and the concentration of cells is greater. Also older cows may have accumulated more intra mammary infections. The analysis of NMR data supports this view. It should be noted that the median values for cell counts by stage of lactation are noticeably lower than the mean averages, suggesting that only a few herds detrimentally affect the average values.

The percentage of herds in each cell count band shows a normal distribution, with most herds at the mid-range values. When comparing herd cell count to the individual animal values most animals are in a lower band suggesting there are a few animals within each herd causing the overall herd average to increase.

CONCLUSIONS

Aggregating data from NMR is a powerful tool for dairy farmers and vets to assess trends in cell counts. By comparing cell count values with management information we can gain a better understanding of what is occurring on the farm.

THE INFLUENCE OF HOUSING SYSTEM ON SOMATIC CELL COUNTS

H.C.F. Wicks[1] and J.D. Leaver[2]

[1]The Agricultural Research Institute of Northern Ireland, Large Park, Hillsborough, Co. Down BT26 6DR
  Email: hannah.wicks@dardni.gov.uk
[2] Royal Agricultural College, Cirencester GL7 6JS

INTRODUCTION

The cost of disease within the dairy industry is associated with loss of yield, discarded milk, cost of treatment and reduced fertility. Mastitis alone is estimated to account for approximately 10% of all cull cows and to have an associated cost of £218 per case (4). In the present study somatic cell count (SCC) records were used to estimate the influence of genetic merit for milk production and environment (housing system) on mastitis.

MATERIALS & METHODS

Some 5618 monthly records of somatic cell counts (recorded from individual cows on commercial farms) were used to estimate the influence of genetic merit (PIN_{95}) and environment on mastitis in dairy cattle on commercial farms. Model 1 was the final model used for somatic cell count data (R^2 = 13%). The somatic cell count (SCC) data were transformed to the natural logarithm to ensure a normal distribution. Environment was described by Housing system.

Model 1:   LN(SCC) = YOC + SOC + MOR + P + S + H + C + G + P*S + C*H + G*H

Where LN(SCC) was the natural logarithm of somatic cell counts; YOC was year of calving, SOC was season of calving, MOR was month of recording, P was parity, S was stage of lactation, H was housing system, C was concentrate intake (kg/d/cow), G was genetic merit (PIN_{95}) and * indicates an interaction.

RESULTS

The mean LN(SCC) increased between year 1 (1997-1998; LN(SCC) = 4.50) and year 2 (1998-1999; LN(SCC) = 4.57) (p<0.05). Autumn calving cows had higher LN(SCC) (4.60) compared with winter calving cows (4.48) (p<0.01). SCC increased around turnout, as lactation progressed and with parity. Somatic cell counts (SCC) were higher for cows housed in straw yards compared with cubicles (Table 1). Although overall there was no significant influence of PIN_{95} on SCC (p=0.23), there was a significant (p<0.05) interaction between genetic merit (PIN_{95}) and housing system and the increase in LN(SCC) with PIN_{95} in straw yards was significant (p<0.05). There was no significant (p=0.38) influence of PIN_{95} on LN(SCC) when cows

were housed in cubicle accommodation. Similarly, the interaction between concentrate feeding (kg/cow/d) and housing system was significant (p<0.001), and while the increase in LN(SCC) with concentrate feeding for cows housed in cubicle accommodation was not significant (p=0.45) the increase in LN(SCC) with concentrate feeding was significant (p<0.001) for cows housed in straw yards. The increase in LN(SCC) per kg concentrate/d averaged 0.082 (± 0.0123).

Table 1   Comparison between straw yard and cubicle housing for somatic cell counts

Parameter	Straw Yard	sem	Cubicle	sem
Geometric Mean (SCC) (x10^3)	123.33		71.15	
Mean (LnSCC)	4.82	0.049	4.26	0.032
LnSCC/PIN$_{95}$	0.0024	0.00106	-0.0007	0.00086
LnSCC/kg concentrate	0.155	0.0181	0.008	0.0111

Where LN(SCC) was the natural logarithm of somatic cell counts (SCC)

DISCUSSION

The higher average SCC for cows housed in straw yard versus cubicle systems suggest a higher incidence of mastitis within straw yard systems. The increase in SCC with genetic merit for cows housed in straw yards is likely to be a result of the longer lying times (3), and higher pathogen challenge, coupled with the presence of larger, slacker teat sphincters in animals producing higher milk yields (2). Larger, slacker teat sphincters may be related to increased milk flow rates. Positive correlations between milk flow rates and SCC have been reported (1). The increase in SCC with level of concentrate feeding may relate to the quantity and consistency of the dung and reduced cleanliness of cows (5).

CONCLUSIONS

Somatic cell counts increased with both genetic merit and concentrate feeding for cows housed in straw yards, but there was no significant influence on SCC for cows housed in cubicle systems.

REFERENCES

1.   Bahr, T., Preisinger, R. & Kalm, E. (1995) Investigations on somatic-cell count and milkability of dairy-cows 2nd communication – genetic-parameters of milkability traits. *Zuchtungskunde* **67***:* 105-116.
2.   Dodd, F.H. & Neave, F.K. (1951) Machine milking rate and mastitis. *J. Dairy Res.* **18**: 240-245.

3.   Fregonesi, J.A. and Leaver, J.D. (2001) Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. *Livestock Prod. Sci..* **68**: 205-216.

4.   Kossaibati, M.A. and Esslemont, R.J. (1995). Daisy Report 4, Wastage in dairy herds. University of Reading.

5.   Ward, W.R., Hughes, J.W., Faull, W.B., Cripps, P.J., Sutherland, J.P. and Sutherst, J.E. (2002) Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. *Vet. Rec.* **151**: 199-206.

EFFECTS OF HABITUATION TO THE MILKING PARLOUR AND COW BREED ON MILK FLOW RATES AND SOMATIC CELL COUNTS IN EARLY LACTATION

H.C.F. Wicks[1], A.F. Carson[1,2], M.A. McCoy[3] and C.S. Mayne[1,2]

[1] The Agricultural Research Institute of Northern Ireland, Large Park, Hillsborough, Co. Down BT26 6DR

[2] Department of Agriculture and Rural Development for Northern Ireland and The Queen's University of Belfast, Newforge Lane, Belfast

[3] Veterinary Sciences Division, Stoney Road, Belfast

INTRODUCTION

During the transition period (defined as three weeks prior to calving to three weeks post-calving) heifers are exposed to physiological, nutritional, management, and social changes as they enter the dairy herd. One obvious change is the introduction of heifers to the milking parlour. Previous work has shown that when mature cows were milked in unfamiliar surroundings, milk yield, milk flow rate and milking duration were affected due to increased stress (3). The objective of the current study was to investigate the effects of habituating heifers to the milking parlour pre-calving on the subsequent somatic cell counts and milk flow characteristics of Holstein-Friesian and Norwegian dairy herd replacements.

MATERIALS & METHODS

Fifty-four spring calving heifers (32 Holstein-Friesian and 22 Norwegian (NRF) dairy cattle) were used to investigate the effects of habituation to the milking parlour prior to calving. The average genetic merit for Holstein-Friesian and Norwegian replacements were 35.9 (s.d. 5.79) PTA_{2000} and 11.7 (s.d. 2.17) Total Merit Index respectively. Animals were grouped according to genotype, predicted calving date, live weight and genetic merit into either of two treatments. Habituation, animals were introduced to the milking parlour (20-point rotary herringbone parlour) 3-weeks prior to calving, whereas Control animals were introduced to the parlour on day of calving. Prior to calving heifers were housed in two groups in adjacent cubicles within the same building. 1 kg/head/d of concentrate was offered in parlour (Habituation) or along the feed passage (Control), all heifers were offered grass silage *ad libitum.* Post-calving heifers were offered 6 kg/head/d concentrate along with grass silage *ad libitum* within a total mixed ration, and 4 kg/head/d concentrate in the parlour, for a 100 day period. The data were analysed using repeated measures REML analysis and fixed effects included week of lactation, breed, treatment and their interactions.

RESULTS

Holstein-Friesian heifers yielded more milk, fat, protein and lactose per day during early lactation than the Norwegian heifers (p<0.001) (Table 1). Milk fat concentration was higher for Holstein-Friesian heifers (p<0.001), but there were no significant differences in milk protein and lactose concentrations between the two breeds. Average and peak milk flow rates were higher for the Holstein-Friesian compared with Norwegian heifers (p<0.001). Somatic cell counts were greater for Holstein-Friesian heifers (Table 1). Heifers in the habituation treatment yielded 1.3 kg/d more milk compared with heifers in the control group (p<0.001), over the first 100 days of lactation. The duration of milking was longer (+0.63 min) and milk flow rates were significantly slower (-0.15 kg/min and –0.29 kg/min average and peak milk flow rates respectively) for heifers in the habituation group compared with the control group (p<0.001). Somatic cell counts were lower for the habituation treatment compared with the control treatment (p<0.001) (Table 1).

Table 1　　**Influence of cow breed and habituation to the milking parlour on milk production, SCC and milk flow rates**

Parameter	H-F	NRF	Control	Habituation	sed
Milk Yield (kg/d)	27.4	24.7***	25.4	26.7***	0.38
Milk Fat (kg/d)	1.096	0.929***	1.003	1.023	0.0207
Milk Protein (kg/d)	0.894	0.801***	0.827	0.867***	0.0121
Milk Lactose (kg/d)	1.379	1.248***	1.284	1.344***	0.0199
$Log_{10}SCC$	1.779	1.669**	1.785	1.663***	0.0366
MFR (kg/min)	2.37	2.17***	2.46	2.20***	0.041

MFR, Milk Flow Rate; ** p<0.0; *** p<0.001

DISCUSSION

The slower milk flow rates recorded in heifers habituated to the milking parlour prior to calving were unexpected, as it has been reported that when mature cows were milked in unfamiliar surroundings milk flow rates were reduced (3). However, the slower milk flow rates may explain the reduction in somatic cell counts in the habituated group as previous authors have reported positive correlations between milk flow rate and somatic cell counts ranging between 0.32 and 0.57 (1). Within-breeds it is well established that there is a positive relationship between milk flow rate and milk yield (1), which may relate to the larger, slacker teat sphincters in animals with high milk yields (2).

CONCLUSIONS

Habituation of heifers to the milking parlour prior to calving has been shown to have positive effects on production and health, in terms of increased milk yield and reduced somatic cell counts.

REFERENCES

1.　Bahr, T., Preisinger, R. and Kalm, E. (1995) Investigations on somatic-cell count and milkability of dairy-cows 2nd communication – genetic-parameters of milkability traits. *Zuchtungskunde* **67***:* 105-116.
2.　Dodd, F.H. and Neave, F.K. (1951) Machine milking rate and mastitis. *J. Dairy Res.* **18**: 240-245.
3.　Rushen, J., Munksgaard, L., Marnet, P.G. and DePassille, A.M. (2001) Human contact and the effects of acute stress on cows at milking. *Appl. Animal Behav. Sci.* **73**: 1-14.

THE EFFECTS OF INOCULATION OF *Mannheimia haemolytica* INTO THE TEAT OF LACTATING EWES

<u>V.S. Mavrogianni</u>[1], G.C. Fthenakis[1], N. Papaioannou[2], I.A. Taitzoglou[1], G. Brellou[2] and P. Saratsis[2]

[1] Faculty of Veterinary Science, University of Thessaly, P.O. Box 199, 43100 Karditsa, Greece

[2] School of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Macedonia, Greece

INTRODUCTION

Mannheimia haemolytica is an important cause of ovine mastitis. It invades the mammary gland through the teat, probably originating from the nasopharynx and tonsils of sucking lambs (2). In previous studies (1, 3) bacteria were inoculated directly into the mammary gland cistern, thus, the early stages of infection, i.e. the invasion and ascent of the organism through the teat, were not studied. Our objective here was to study the outcome of challenging ewes with *M. haemolytica,* at different sites on the teats.

MATERIALS AND METHODS

Two *M. haemolytica* isolates, one (A) from a case of clinical mastitis in a ewe and the second (B) from the teat duct of a clinically healthy ewe, were used for the inoculations. Thirty-two ewes, divided into eight subgroups (n=4), were included into the experiment. Animals of each subgroup were inoculated with ~1200 c.f.u of one or other of the isolates (i) directly into a gland cistern or (ii) 6 mm into a teat duct or (iii) 2 mm into a teat duct or (iv) had one teat immersed in 40 ml of a bacterial broth culture twice for 1 minute on two consecutive days.

Regular clinical examinations of the mammary glands and teats were carried out before and after challenge; secretion samples were collected for bacteriological, CMT and Giemsa examinations. The external surface of the teats of subgroup (iv) was swabbed with cotton.

The ewes in each subgroup were euthanatised 2, 4, 7 and 11 days after challenge and the mammary glands and teats were dissected. Scrapings from the teat duct and teat cistern, as well as tissue samples from the mammary gland, were obtained and cultured. Tissue samples from the mammary gland and the teats were obtained for histopathological examination.

RESULTS

Before challenge, all mammary glands and teats of all ewes were healthy, as confirmed by the results of clinical, bacteriological and cytological examination. All ewes challenged intramammarily (sub-groups Ai, Bi) developed clinical mastitis, as confirmed by the results of clinical, bacteriological and cytological examination. Histological lesions characteristic of *M. haemolytica* mastitis (neutrophilic infiltration, extravasation) were evident.

None of the ewes whose teat was submerged in the *M. haemolytica* broth culture (sub-groups Aiv, Biv) developed mastitis. The bacteria were recovered from the external teat surface for up to five days after challenge, they were also isolated from the teat duct three days after challenge. No histological lesions were seen in sections of teat duct or the mammary parenchyma.

The ewes inoculated with *M. haemolytica* 6 mm or 2 mm into the teat duct (subgroups Aii, Bii and Aiii, Biii, respectively) developed subclinical mastitis -confirmed by the bacteriological, cytological and histopathological evidence by two days after challenge. Leucocytic infiltration was also evident in histological sections of teat the duct.

DISCUSSION

The *M. haemolytica* isolate, recovered from the teat duct of a healthy ewe, caused clinical mastitis when it was deposited directly into the cistern of the mammary gland, i.e. after the defense mechanisms of the teat had been overridden. This suggests that the defense mechanisms of the teat were initially limiting the growth of the organism. When these were by-passed the organism exhibited its full pathogenic potential and caused mastitis.

Neither *M. haemolytica* isolate caused clinical mastitis when deposited inside the teat duct. The leucocytes seen in histological sections could have had a role in limiting the infection and protecting the mammary gland, however, other factors (e.g. defense mechanisms of the teat) might also be involved. These findings confirm that the teat duct acts to protect the mammary gland against invasion by these bacteria.

The isolation of *M. haemolytica* from inside the teat duct of ewes whose teats had been submerged into a broth culture further supports the hypothesis that in cases of mastitis the organism was likely to originate from the sucking lambs (2). However, as no mastitis was induced, other factors possibly enhance the ability of the organism to by-pass the defences of the teat.

REFERENCES

21. El-Masannat, E.T.S., Jones, J.E.T. and Scott, M.J. (1991) The experimental production of mastitis in sheep by intramammary inoculation of *Pasteurella haemolytica.* mastitis. *J. comp. Path.* **105**: 455-65.

22. Scott, M.J. and Jones, J.E.T. (1998) The carriage of *Pasteurella haemolytica* in sheep and its transfer between ewes and lambs in relation to mastitis. *J. comp. Path.* **118**: 359-63.

23. Watkins, G.H. and Jones, J.E.T. (1992) The effect of the intramammary inoculation of lactating ewes with *Pasteurella haemolytica* isolates from different sources. *J. comp. Path.* **106**: 9-14.

PRELIMINARY RESULTS OF A STUDY ON PAIN ASSESSMENT IN CLINICAL MASTITIS IN DAIRY COWS

M.H. Milne[1], A.M. Nolan[1], P.J. Cripps[2] and J.L. Fitzpatrick[1]

[1] Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden, Glasgow G61 1QH

[2] Department of Veterinary Epidemiology, Leahurst, University of Liverpool Veterinary School, Chester High Road, Wirral CH64 7TE

SUMMARY

Recognition, alleviation and control of pain and stress are central to ensuring good welfare in food producing animals. Over 100 dairy cows with clinical mastitis (mild or moderate) were followed to assess pain associated with clinical mastitis. On the day of diagnosis of clinical mastitis, the average heart rate, respiratory rate and rectal temperature were significantly higher in cows with moderate mastitis compared to cows with mild mastitis and compared to normal cows. Pain thresholds, measured using a mechanical device, were significantly lower in cows with mild and moderate mastitis compared to normal cows, indicating the presence of mechanical hyperalgesia. The hock-hock distances were significantly higher in cows with mild or moderate mastitis compared to normal cows, indicating that cows with mastitis alter their stance.

INTRODUCTION

Mastitis is a major disease problem for the dairy industry, causing significant economic losses and adverse cow welfare. Inflammation induces alterations in normal pain information processing, which may have serious consequences for the animal. These include allodynia (perception of innocuous stimuli as noxious) and hyperalgesia (exaggerated response to noxious stimuli) that leads to pain hypersensitivity. A pilot study in cows with mastitis indicated that acute mastitis was associated with mechanical hyperalgesia (1). This larger study was undertaken to assess further the use of a range of parameters in assessing pain in cows with mastitis.

MATERIALS & METHODS

Commercial dairy cows were recruited onto the study on the day of diagnosis of 'mild' or 'moderate' clinical mastitis. Clinical mastitis was classed as 'mild' when there were changes in milk appearance but the udder was normal, and 'moderate' when there were changes in milk appearance and the udder was hot, swollen or painful to touch, but the cow was not 'unwell' or requiring systemic antibiotic therapy. Normal cows were recruited as controls. All cows were examined clinically (including measurement of heart and respiratory rates and rectal temperature) and milk samples were collected on the day of diagnosis. The distance between the hocks was

measured as a proxy indicator of altered cow stance. Response thresholds to mechanical stimuli were measured on each hindlimb following the clinical examination using a modification of the method described earlier (2). Results were expressed as the difference between the control leg (unaffected side) and the ipsilateral leg (affected side). Kruskal-Wallis and one-way ANOVA tests in Mintab Statistical Software (Minitab Inc.) were used to compare parameters from mild and moderate cases of mastitis and normal cows.

RESULTS

One hundred and seventeen cows with clinical mastitis and 45 normal cows were studied. Sixty-one of the clinical cases of mastitis were mild and 56 were moderate. The hock-hock distance, individual somatic cell count (IQSCC) and protein content of the milk of normal animals were lower compared to cows with mastitis (both mild and moderate cases) (p<0.001). The mechanical pain threshold difference and lactose content of milk were higher in normal animals compared to cases with mastitis (both mild and moderate) (p<0.001). The heart rates, respiratory rates and rectal temperatures of cows with moderate mastitis were higher (p<0.001) compared to cows mild mastitis and normal animals.

There was no difference in days calved, age of cows, and milk yield between the groups (p>0.07). The bacteriological results showed that in 28% of moderate cases *Escherichia coli* were isolated and in 39% *Streptococcus uberis* were isolated compared to 16% and 18%, respectively for *E. coli* and *S. uberis*, in mild cases.

DISCUSSION

The results suggest that cows with mild and moderate mastitis exhibit mechanical hyperalgesia, indicating altered pain information processing as a consequence of the inflammatory disease. These results indicate that this technique can be used to indirectly monitor pain in cattle with mastitis. Furthermore, the response to analgesic treatments such as the non-steroidal anti-inflammatory drugs, which have known anti-hyperalgesic properties, can be measured. It is interesting to note that more of the cases of moderate mastitis were likely to be due to *E . coli* or *S. uberis* compared to mild cases.

ACKNOWLEDGEMENTS

REFERENCES

1.   Fitzpatrick, J.L., Young, F.J., Eckersall, D., Logue, D.N., Knight, C.H. and Nolan, A. (1999) Mastitis – A Painful Problem? *Cattle Practice* **7**: 225-226

2.   Nolan, A., Livingston, A., Morris, R. and Waterman, A. (1987) Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. *J. Pharmacol. Methods* **17**: 39-49.

MULTILOCUS SEQUENCE TYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CASES OF BOVINE MASTITIS

E.M. Smith, L.E. Green, G.F. Medley, H.E. Bird and C.G. Dowson
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL

SUMMARY

Multilocus sequence typing was used to type a collection of 191 *Staphylococcus aureus* isolates collected from a Somerset organic dairy farm.

The longitudinal sampling regimen employed in this study showed that two closely related strains predominated on the farm throughout the sampling period, the more dominant strain however, changed over time.

Strain typing also showed that isolates of *S. aureus* display a level of host specificity, and that virulence may also be linked to a strain's underlying genetic make-up.

INTRODUCTION

In the early 1990s environmental *S. aureus* isolates were detected on a dairy farm (1, 2), leading the investigators to conclude that *S. aureus* was 'ubiquitous in the dairy farm environment' (2). However, little strain typing was carried out and disease severity was not investigated.

Little is known about the potential effects of environmental reservoirs of *S. aureus* in this country, or about the numbers and distribution of strains of *S. aureus* on and between farms, and the effects these may have on disease severity. A collection of isolates was made from a Somerset organic dairy farm experiencing an outbreak of *S. aureus* mastitis to help answer some of these questions.

MATERIALS & METHODS

Monthly quarter milk samples were taken from 26 cows over a ten-month period. Dairy cow environment samples were also collected to determine the on-farm *S. aureus* population.

Speciation of isolates were confirmed by polymerase chain reaction (PCR) and all confirmed *S. aureus* isolates were then typed using multilocus sequence typing (MLST).

A selection of *S. aureus* isolates was donated by Dr. A. Bradley (Bristol University) for comparison with the isolates collected in this study.

RESULTS

Sample collection

A total of 2185 samples were collected, including 959 (43.9%) quarter milk samples. Of the 1681 samples analysed for *S. aureus*, 155 (9.2%) contained coagulase-positive staphylococci. These 155 samples yielded 196 isolates, 191 (97.4%) of which proved to be *S. aureus*.

Multilocus sequence typing (MLST)

All 191 isolates were typed using MLST, resulting in the discovery of two novel alleles and five new sequence types (ST). The strains donated by A. Bradley yielded five novel alleles and one new ST.

DISCUSSION

While a number of strains of *S. aureus* were found on the farm, one or two (in this case closely related) strains predominated. The results also indicate a transition at the herd level from one dominant strain to another over time, but further work is required to confirm this is the case.

The strain typing evidence also suggests that there is some difference between human and bovine infecting strains, including those isolated from farm workers, and between those causing clinical and sub-clinical disease.

CONCLUSIONS

- ➢ One or two strains predominated in the herd
- ➢ Human and bovine strains show host specificity
- ➢ Virulence seems to be linked to strain type

ACKNOWLEDGEMENTS

REFERENCES

1.   Matos, J.S., White, D.G., Harmon, R.J. and Langlois, B.E. (1991) Isolation of *Staphylococcus aureus* from sites other than the lactating mammary gland. *J. Dairy Sci.* **74**: 1544-1549.

2.　　Roberson, J.R., Fox, L.K., Hancock, D.D., Gay, J.M. and Besser, T.E. (1994) Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. *J. Dairy Sci.* **77**: 3354-3364.

PRELIMINARY RESULTS FROM A QUESTIONNAIRE ON TEAT CLEANING AND PARLOUR HYGIENE

R.G. Protheroe[1], L.A. Sinclair[2], C.M. Brizuela[2], T.J. Hocking[1], E. Atkinson[1], H. Worton[1] and H. Gibson[1]

[1] University of Wolverhampton, School of Applied Sciences, Wulfruna Street, Wolverhampton WV1 1SB

[2] Harper Adams University College, Department of Animal Production and Science, Newport, Shropshire TF10 8NB

INTRODUCTION

A research proposal to study the effectiveness of pre-milking teat cleaning methods, prompted by wider concerns about milk quality, was developed in conjunction with the Food Standards Agency. A questionnaire was used to establish baseline information about current teat cleaning and other aspects of milking practice and herd management. Further study by observations of milking and bacteriological assay, will follow analysis of the questionnaire results. Some preliminary results from the questionnaire are presented.

PRELIMINARY RESULTS

A questionnaire was sent to 1000 dairy farms to establish up-to-date information on the range of parlour types on dairy farms and the current pre-milking teat cleaning practices in England and Wales.

Information central to the study on pre-milking teat cleaning and milk quality were: milking parlour type, pre-milking teat cleaning practices, average annual bulk tank total bacterial count (AATBC), average annual somatic cell count (AASCC)

The predominant parlour type was herringbone (75%), followed by abreast (17%), pipeline (2%), rotary (2%) Other types (shippon, tandem, fast exit, tribone, inline, and robotic) totalled only 4%. Pre-milking teat cleaning was carried out by 96% of respondents. The predominant method used was dry wiping (43%), followed by medicated wiping (19%), dipping (12%), wet cloth (11%), spray (8%), other methods (3%).

Linear regression indicated no significant relationship between TBC and SCC, therefore represent separate variables, which may respond differently to individual hygiene practices. Subsequent farm practice observation and teat sampling will establish the reliability of information supplied by the questionnaire and determine the microbiological challenge on teat surfaces and the extent of teat contamination.

ACKNOWLEDGEMENTS

THE EFFECT OF DIFFERENT DRY COW THERAPY TREATMENTS ON MILK QUALITY IN A DAIRY HERD UNDERGOING ORGANIC CONVERSION

E.K.S. Green, J.F. Robertson and E.J. Allan
School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland AB24 5UA

SUMMARY

Fifty-one pedigree Holstein dairy cows were allocated to one of three dry period treatments; antibiotics, an organic alternative or an external teat sealant. Milk samples were taken on three occasions and analysed for quality. Antibiotics were the most effective at keeping somatic cell count (SCC) low, although all SCC remained low. The organic alternative significantly reduced *Streptococcus* isolates. The sealant was the least effective.

INTRODUCTION

In organic dairy farming, the reduction in use of prophylactic medicines requires positive health management. This can be supported by the use of alternative treatments, e.g. herbal or homeopathic preparations. In conventional dairying dry cows rely on antibiotics to protect against mastitis infections. It is, therefore, vital that suitably effective alternatives are found. This trial involved the use of three different preventative treatments and recorded the resulting effect on cow health and milk quality.

MATERIALS AND METHODS

The trial began in June 2002 and was completed in April 2003. Fifty-one animals, selected according to their calving date, were randomly allocated to one of three treatment groups. Treatments applied at drying off were:

Abbreviation	Treatment	Product
AB	Antibiotics	Cepravin, Schering-Plough
Org	Organic alternative therapy	Cinnatube, Nutragena
Seal	Sealant	Dryflex, DeLaval Ltd

Duplicate sets of 25 ml quarter milk samples were taken directly from the teat by hand, into sterile tubes and transported at <5°C, following a strict protocol, on three occasions:

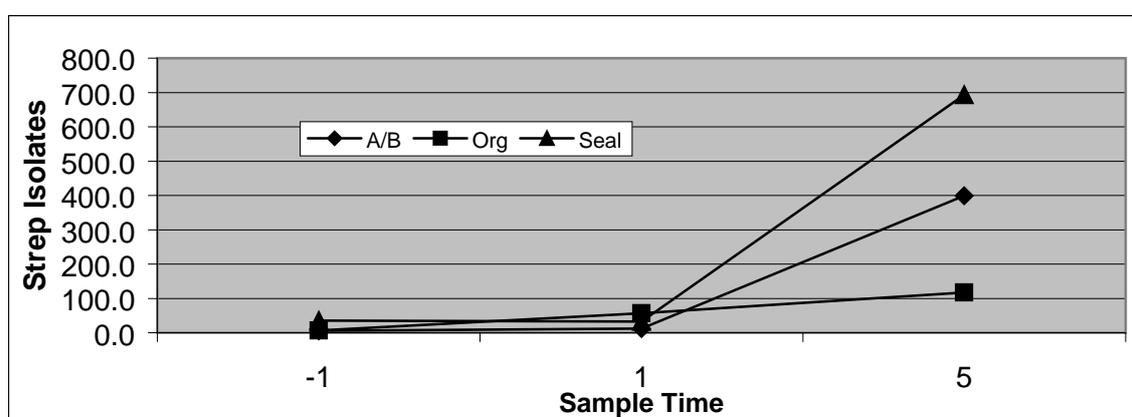| 1	One week before drying off	Period -1
2	One week after calving	Period +1
3	Five weeks after calving	Period +5

One set of samples was analysed for SCC. The other sample was analysed for Total Viable Count (TVC), Streptococcus *spp.*, Staphylococcus *spp.* and coliforms. Animals were monitored during the dry period every three days for signs of mastitis. A scoring system that used 0 as an indication of no infection rising to 3 for clinical mastitis was devised to indicate the level of any infection.

RESULTS

 ➢ All trial animals consistently scored zero (no infection) during the dry period, and no animals were clinically infected with mastitis during the trial period.

 ➢ Period had a significant effect on Strep *spp.* ($p<0.001$), and Staphs ($p<0.01$).

 ➢ The Org treatment significantly reduced Strep isolates ($p<0.001$) at period +5 (x =117.2) compared with the other treatments (AB x =398.7, Seal x = 693.4) (Figure 1)

 ➢ Cows allocated to the sealant group had significantly increased TVC ($p<0.05$) before treatment but not in periods +1 and +5.

 ➢ Mean group SCC were highest at period +1 for the Org group ($p<0.001$). SCC were reduced by period +5, with no significant differences between treatments.

 ➢ The incidence of mastitis treatment in the main herd was 38/120 (32%), during the trial period.

Figure 1 shows the mean Strep. isolates per group. All +5 samples were taken from September onwards when Strep. populations tend to increase (1)

Figure 1   Mean streptococcal isolations per trial group (n=204)

DISCUSSION AND CONCLUSIONS

The teat sealant performed less well than the other treatments. Applied to teats for one week at the beginning and end of the dry period, it does not provide adequate protection for the full dry period. Sealant would be an ineffective alternative to antibiotics. The organic product was effective in the third period at lowering SCC and Strep. numbers. This appears to be an alternative to antibiotics. The antibiotics kept the SCC values consistently low. The low incidence of mastitis (0/51) for the trial group may reflect attentive management, but the main herd reflects results from a separate six-year study that showed up to 27% of a converting herd developed mastitis (2).

ACKNOWLEDGEMENTS

REFERENCES

1.   Waage, S., Mørk, T., Rørros, A., Assland, D., Hunshamar, A. and Ødegaard, A. (1999) Bacteria associated with clinical mastitis in dairy heifers. *J. Dairy Sci.* **82**: 712-719.
2.   Weller, R.F. and Davies D.W.R. (1998) Somatic cell counts and incidence of clinical mastitis in organic milk production. *Vet. Rec.* **143**: 365-366.

MASTITIS TRENDS IN UK DAIRY HERDS: 1998-2003

Alastair Macrae, David Whitaker, Liz Burrough and Jim Kelly

Dairy Herd Health and Productivity Service, Division of Veterinary Clinical Studies, R(D)SVS, University of Edinburgh, EBVC, Easter Bush, Roslin, Midlothian, EH25 9RG

Website: http://www.vet.ed.ac.uk/dhhps/

E-mail: A.I.Macrae@ed.ac.uk

SUMMARY

Disease and disposal rates were obtained for a large number of dairy herds in the UK from 1998-2003. Although the average disposal rate remained relatively constant at approximately 22%, the disposal rate for mastitis rose slightly from 3.6% in 1998/1999 to 5.1% in 2002/2003. Over the same period, the annual clinical mastitis rate rose from 36.0% to 47.0% and the bulk milk somatic cell count (BMSCC) rose from 140,000 cells/ml to 179,000 cells/ml. Housing cows in straw yards was associated with a significantly higher annual clinical mastitis rate and annual mean BMSCC than housing cows in cubicles. However, the best 25% of herds consistently achieved excellent mastitis figures, suggesting that application of existing knowledge and control methods can achieve satisfactory results.

INTRODUCTION

There are few studies using large numbers of dairy herds in the UK to show trends in the rates of mastitis and associated factors (1, 2). The Dairy Herd Health and Productivity Service records monthly data on the disease and disposal rates on a large number of dairy herds for a dairy farm quality assurance scheme (White Gold service), predominantly in the South of England (3). This poster presents the analysis of this dataset. Mastitis rate was measured as the number of cow cases (1), and BMSCC was obtained from milk purchaser receipts.

RESULTS

Examination of 219 herds with continuous data available through 1998 to 2001 revealed very similar figures to those in Tables 1 and 2. Analysis was performed using Pearson's correlation coefficient, which demonstrated a positive association between herd size and annual mean BMSCC, and BMSCC and annual mean clinical mastitis rate. However, there was no association between herd size and annual mean clinical mastitis rate.

Table 1   Disposal rates in UK dairy herds (1998-2003)

Season	1998/1999	1999/2000	2000/2001	2001/2002	2002/2003*
Number of herds	445	484	341	248	187
Herd size	132 (92-161)	144 (100-176)	153 (109-184)	163 (116-197)	156 (102-191)
Annual disposal rate (%)	21.8 (15.9-26.3)	24.8 (17.9-29.9)	21.4 (15.0-25.6)	21.7 (14.1-26.9)	21.8 (16.1-26.8)
Disposal rate for mastitis (%)	3.6 (0.9-4.8)	4.6 (1.4-6.5)	4.1 (1.3-5.8)	4.4 (1.1-6.5)	5.1 (2.0-6.7)

Mean values (range from lowest 25% to highest 25% of herds) shown.
* indicates that dataset for season 2002/2003 is incomplete.

Table 2   Mastitis trends in UK dairy herds (1998-2003)

Season	1998/1999	1999/2000	2000/2001	2001/2002	2002/2003*
Clinical mastitis rate (%)	36.0 (19.6-48.3)	36.7 (20.7-48.6)	39.6 (22.5-50.1)	42.8 (22.9-55.2)	47.0 (23.1-63.6)
Annual BMSCC ('000 cells/ml)	140 (104-169)	147 (114-176)	157 (120-191)	174 (135-206)	179 (142-209)

Mean values (range from best 25% to worst 25% of herds) shown.
* indicates that dataset for season 2002/2003 is incomplete.

Influence of housing environment on clinical mastitis rate and BMSCC

Herds were separated into those housed exclusively over winter in straw yards and those housed exclusively in cubicles. Cows housed in straw yards had a significantly higher annual mean clinical mastitis rate and annual mean BMSCC than cows housed in cubicles in all 5 years. However, some of the best data were found for cows housed in straw yards and some of the worst data were found with cubicles, suggesting that management rather than the actual system plays a crucial role in the incidence of mastitis.

CONCLUSIONS

These results are similar to other independent studies (1, 2), and indicate that the average annual rate of clinical mastitis has increased slightly in the period 1998-2003. During this same period, the disposal rate for mastitis and the mean annual BMSCC have also risen. At present, this database provides the largest amount of data on current disease and disposal trends

in the UK dairy industry, and enables the setting of attainable targets to control mastitis, improve economic returns and advance welfare standards in British dairy herds. The wide variation in mastitis rates suggests that excellent mastitis control is achievable with the use of current knowledge.

ACKNOWLEDGEMENTS

REFERENCES

1.   **Esslemont, R.J. and Kossaibati, M.A. (2002) The costs of poor fertility and disease in UK dairy herds. Intervet UK, Milton Keynes.**

2.   Peeler, E.J., Green, M.E., Fitzpatrick, J.L. and Green, L.E. (2002) Study of clinical mastitis in British dairy herds with bulk milk somatic cell counts less than 150,000 cells/ml. *Vet. Rec.* **151**: 170-176.

3.   Whitaker, D.A., Kelly, J.M. and Smith, S. (2000) Disposal and disease rates in 340 British dairy herds. *Vet. Rec.* **146**: 363-367.

COMPARISION OF THE EFFICACY OF A TEAT-DISINFECTANT APPLIED AS A FOAM OR AS A LIQUID DIP USING AN *IN VITRO* TEST

J. Cooper[1], A. Beggin[2] and J.E. Hillerton[1]

[1] Institute for Animal Health, Compton Laboratory, Newbury, Berks RG20 7NN
[2] Ambic Equipment Limited, Avenue Four, Station Lane, Witney, Oxon OX28 4XT

SUMMARY

An *in vitro* test was used to investigate the relative efficacies of a teat-disinfectant applied as either a foam or a liquid dip against a combination of three mastitis-causing bacterial species (*Staphylococcus aureus*, *Escherichia coli* and *Streptococcus uberis*). The test involved using strips of pig-skin (n=20) to imitate the skin surface of the udder. The strips were inoculated with bacterial cultures followed by treatment with water or the foamed test product or the liquid test product. Following a standard exposure time the numbers of surviving bacteria were estimated.

The product was more effective at reducing bacterial counts when applied as a foam than as a dip against two of the three species investigated. Treatment with foam reduced *S. uberis* counts relative to treatment with disinfectant dip but not significantly differently. Overall, logarithmic bacterial counts following treatment with foam were approximately 50% lower than following treatment with the liquid dip.

INTRODUCTION

Teat disinfectants are conventionally applied as a liquid dip but can also be applied as a foam by means of a foaming teat cup (Ambic Equipment Limited, UK). It was not known whether application by foam is as effective at killing mastitis-causing bacteria as application by dipping. Hence, the effectiveness of two methods of application was assessed using a novel *in vitro* test. Bacterial preparations applied to strips of pig-skin were used to mimic the surface of the udder, allowing the experiment to be conducted under controlled laboratory conditions. This alternative approach provided meaningful data without the need to use experimental animals.

MATERIALS & METHODS

A standard volume of a bacterial suspension containing between 2×10^5 and 8×10^5 cfu of each of three mastitis causing species (*S. aureus, E. coli* and *S. uberis*), was applied to sterile strips of pig skin. Strips were then briefly immersed in a commercially available teat disinfectant either a liquid or foam, or in water, and left to dry for a further 30 minutes.

Bacterial counts from each sample were then obtained by rinsing the strips in a disinfectant neutraliser and measuring colony forming units of bacteria after culture on selected agar media.

RESULTS

Both dip and foam treatments resulted in a marked reduction in bacterial counts for all three species tested relative to the water treated controls. Treatment of the samples with disinfectant foam resulted in significantly fewer *S. aureus* and *E. coli* than treatment with disinfectant dip. Treatment of the samples with disinfectant foam appeared to reduce *S. uberis* relative to treatment with disinfectant dip but this difference was not statistically significant. On average, logarithmic counts were approximately 1.8, 2.3 and 1.8 times higher after treatment with dip than with foam for *S. aureus*, *E. coli* and *S. uberis* respectively.

DISCUSSION

Under the test conditions, treatment with foam resulted in lower bacterial counts than treatment with liquid dip for all three bacterial species and a statistically significant difference was demonstrated for two of the three bacteria used. Overall, treatment with the foam resulted in an average decrease in logarithmic bacterial count of approximately 50% relative to the liquid dip treatment.

Application of the product as a foam used a smaller volume of the product than application as a liquid dip and so may be economically beneficial. The results of this study suggest that such benefits would be achieved without compromising the effectiveness of the product.

CONCLUSIONS

During an *in-vitro* test, application of a foamed teat disinfectant was more effective in reducing bacterial counts on a skin surface of three mastitis causing species than when the same product was applied as a liquid dip.

VARIATION IN THE BULK RUBBER CHEMISTRY AND DYNAMIC MECHANICAL PROPERTIES OVER THE LIFE OF A LINER

M. Hale[1], D. Boast[1], J.E. Hillerton[2], N. Middleton[2] and I. Ohnstad[3]
[1] Avon Rubber, Melksham, Wilts
[2] Institute for Animal Health, Compton, Newbury, Berks RG20 7NN
[3] ADAS, Taunton

The chemical and mechanical properties of the milking liner have been examined as part of a study of their deterioration with age. 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 chemical composition and dynamic mechanical properties of liners were examined at different positions down the bore of liners of various ages. The internal surface of liners was examined at different ages.

The plasticiser in the rubber (DOP) appears to be extracted as butterfat is absorbed into the liner with age. This appears to happen locally, being maximal 30-55 mm from the top of the liner. 6 PPD protects the rubber from degradation, it is also extracted.

Tan delta, the ratio of energy adsorbed (viscous modulus) to the elastic modulus, decreases at a steady rate as liners age. Again, this is a local effect. The major changes occur from 2300 to 4300 milkings.

Nitrile rubber swells in butterfat but does not swell significantly in fresh milk so some mechanism is needed to explain the adsorption of butterfat. Matching the contact speed and pressure to milking conditions, using a rolling test of liner rubber in a bath of milk, results in a greasy deposit on the rubber surface. Mechanical attrition of milk between the rubber and teat is likely to break down the fat emulsion of milk so that the butterfat can be adsorbed into the rubber.

CLUSTER ALIGNMENT – GET IT RIGHT!

Tim McKendrick
ADAS Dairy Group, New Agriculture House, Blackbrook Park Avenue, Taunton TA1 2PX
Email: tim.mckendrick@adas.co.uk

INTRODUCTION

Cluster alignment is a major influence on the effectiveness of a milking unit. Cluster alignment can vary enormously from farm to farm and even on the same milking machine. The poster demonstrates the variation in cluster alignment found on farms and the potential adverse effects that can result from poor alignment. Examples of satisfactory and poor cluster alignment are illustrated.

CAUSES OF POOR CLUSTER ALIGNMENT

➢ Cluster twisting – twisted long milk/pulse tubes, poorly aligned swing arms or milking points, no cluster/long milk tube support arm/device or kerb clip, length of the long milk tubes, insufficient room in the standing. Low level plants can often be more prone to cluster twisting, if support is not provided for the correct alignment of the long milk tube.

➢ Cluster tilting – Udder confirmation, cluster weight, weight distribution of the cluster, length of the long milk tubes.

EFFECTS OF POOR CLUSTER ALIGNMENT

➢ Reduced milk yield – ineffective milking out

➢ Increased incidence of liner slip

➢ Increased cell count and incidence of clinical mastitis

➢ Reduced milking speed

➢ Increased proportion of light quarters

➢ Reduced cow comfort and poorer cow behaviour

OPERATOR INFLUENCE

In certain circumstances, poor cluster position can be improved by an attentive careful operator, who is willing to adjust units during milking.

IMPROVEMENTS

Methods for improving cluster alignment are often simple and cheap. In only some instances can satisfactory cluster alignment be achieved by carrying out major changes to the milking equipment.

METHODS OF IMPROVING CLUSTER ALIGNMENT

- ➢ Adjustments made by the milker, where required, during milking
- ➢ Correct length of long milk and pulse tubes to prevent drag on the cluster
- ➢ Simple metal hook attached to the lower rump rail to improve long milk tube alignment.
- ➢ Hook or clip on the long milk tube to attach to the kick kerb.
- ➢ Provide adequate standing width, in some parlours old feeders replaced by new wall mounted ones can provide extra standing width.
- ➢ Fitting of swing over arms, or cluster support arms
- ➢ Care at the point of installation to ensure equipment is installed with cluster alignment in mind