

2011 BRITISH MASTITIS CONFERENCE

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Topics:

- **Teat preparation**
- **Research updates**
- **Future of antimicrobials**
- **Large herds**

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Wednesday 2nd November 2011

**Pitch View Suite, Worcester Rugby Club,
Sixways Stadium, Warriors Way,
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BMC 2011

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GENERAL INFORMATION

TIMETABLE of EVENTS

09:00	ARRIVE / REGISTRATION / COFFEE and POSTER DISPLAY	
09:45	CHAIRMAN'S INTRODUCTION	Ian Ohnstad <i>The Dairy Group, UK</i>
	Controlling Somatic Cell Counts	
09:55	Teat preparation – what are the benefits to milk quality, udder health and milking performance?	Morten Dam Rasmussen Aarhus University, Denmark
10.55	Questions and Discussion	
11:10	COFFEE and POSTERS	
	Research updates	
11:40	PCR versus Bacteriology: detection of mastitis pathogens by real-time PCR in clinical and subclinical mastitis samples	Brian Pocknee DHC, UK
11:55	The role of mastitis vaccines	Anja Rothkamp vaxxinova GmbH, Germany
12:10	Influence of genetics on mastitis	Jon Reader Synergy Farm Health, UK
12:25	Mastitis and infertility: preliminary results of a large retrospective study	John Cook Genus ABS, UK
12:40	Questions and Discussion	Chris Hudson University of Nottingham, UK
13:00	LUNCH and POSTERS	
14:10	CHAIRMAN'S INTRODUCTION and VOTING ON POSTERS	Elizabeth Berry DairyCo, UK
	Milking management	
14:15	Antimicrobials – past, present and future	Jenny O'Connor MSD Animal Health, UK
14:45	Questions and Discussion	
14:50	Large herds – a farmer's view	Nick Cobb Dorset, UK
15:20	Mastitis in large herds – a vet's view	Mark Burnell Synergy Farm Health, UK
15:50	Questions and Discussion	
16:00	POSTER AWARD and CLOSE	
16:15	TEA and DEPART	

TABLE of CONTENTS (Continued)

General information:

Timetable of events	i
Table of contents (paper titles)	ii
Table of contents (poster titles)	iii
Chairman’s introduction	iv
Further information	v
Sponsors	vi
NMC	vii

Scientific programme:

Teat preparation

Teat preparation – what are the benefits to milk quality, udder health and milking performance? Morten Dam Rasmussen, Aarhus University, Denmark	1 - 7
---	-------

Research Updates

PCR versus Bacteriology: detection of mastitis pathogens by real-time PCR in clinical and subclinical mastitis samples Anja Rothkamp, vaxxinoa GmbH, Germany	9 - 16
---	--------

The role of mastitis vaccines Jon Reader, Synergy Farm Health, UK	17-25
--	-------

Influence of genetics on mastitis John Cook, Genus ABS, UK	27-35
---	-------

Mastitis and infertility: preliminary results of a large retrospective study Chris Hudson, University of Nottingham, UK	37-42
--	-------

Antimicrobials

Antimicrobials – past, present and future Jenny O’Connor, MSD Animal Health, UK	43-51
--	-------

Large herds

Mastitis in large herds – a vet’s view Mark Burnell, Synergy Farm Health, UK	53-56
---	-------

Poster abstracts (presenting author underlined):

- Use of the 3M™ Clean-Trace™ Surface ATP Test to assess parlour hygiene and investigate Bactoscan breakdowns** 57-58
J Roberts and M Haslam
Lambert Leonard & May LLP, Broughall, Whitchurch, Shropshire, UK
- Bacterial migration through the teat canal related to liner action** 59
D Forbes¹ and W Gehm²
¹Animal Health and Veterinary Laboratories Agency, New Haw, Addlestone, KT15 3NB
²9502 NYS Rt 79Lisle, New York 13797, USA
- Strategies to reduce milking duration of individual cows** 61-62
P Edwards and J Jago
DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand
- Monitor the level of chronic high cell count cows to avoid penalties** 63-64
J Hanks
PAN Livestock Services Ltd, SAPD, Earley Gate, P.O.Box 237, Reading, UK
- Mastitis bacteriology and qPCR – Myths and realities** 65-66
A J Bradley¹ and A Biggs²
¹Quality Milk Management Services Ltd, Unit 1, Lodge Hill Industrial Park, Station Road, Westbury-sub-Mendip, Nr Wells, Somerset, UK; ² Vale Veterinary Centre, The Laurels, Station Road, Tiverton, Devon, UK
- Associations between herd size and somatic cell count for Irish and UK dairy herds**
S C Archer^{1,2}, **F McCoy**², **F Buckley**², **W Wapenaar**¹, **M J Green**¹
¹University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington Campus, Sutton Bonington, Leicestershire, UK 67-68
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Appendix

National Mastitis Survey 2011

CHAIRMAN'S INTRODUCTION

Welcome to the 23rd British Mastitis Conference. Following overwhelming feedback from last year's delegates, we find ourselves back at the modern conference facilities at Worcester Rugby Club.

The Organising Committee has worked hard throughout the year to bring together a group of speakers, both international and home grown, that we believe will prove thought provoking and stimulating.

Our first paper examines the role of teat preparation and its benefits in terms of milk quality, udder health and milking performance.


We then move into our research updates with four papers considering PCR and bacteriology, the role of mastitis vaccines, the influence of genetics on mastitis and the link between mastitis and infertility. These four papers are followed by an opportunity for delegates to debate with the presenters.

After lunch, we will turn our attention to Antimicrobials taking a look at the past and the present but more importantly looking forward to the role of antimicrobials in the future. The conference is closed with two papers examining the management of mastitis at the farm level. We will hear about mastitis management from the perspective of both the farmer and the veterinary surgeon.

As always we have an excellent group of posters and I would urge you all to make time to review the posters and speak with the authors.

We continue to try to find you the best speakers with the best and most relevant (and latest) information. This is achievable only thanks to all our generous sponsors. This year our sponsors are: Ecolab, Boehringer Ingelheim, MSD Animal Health, Evans Vanodine, Vetoquinol, Lely and Kilco. As usual the event could not happen without able administration, now provided by Karen Hobbs and Anne Sealy at *The Dairy Group*.

Finally, as always, thank you for attending and supporting the conference. I trust you will have an enjoyable and worthwhile day.



Ian Ohnstad
British Mastitis Conference Chairman
The Dairy Group

FURTHER INFORMATION

Organised by *The Dairy Group*, DairyCo and University of Nottingham

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Nottingham

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A global organization for mastitis control and milk quality

The NMC is a professional organization that promotes research and provides information to the dairy industry to help reduce mastitis & enhance milk quality. For nearly 50 years, the NMC has distinguished itself internationally as a leader in meeting those objectives.

What does NMC do?

- Provides a forum for the global exchange of information on mastitis and milk quality
- Publishes educational materials including books, brochures and CDs
- Establishes guidelines for mastitis control and milking management practices
- Monitors technological and regulatory developments relating to udder health, milk quality and milk safety
- Conducts meetings & workshops, providing educational opportunities for all segments of the dairy industry
- Helps fund the National Mastitis Research Foundation

*A commitment to
reducing mastitis and
enhancing milk quality*

Who are the members of NMC?

NMC membership is comprised of people from more than 40 countries, representing a wide range of dairy professionals who share an interest in milk quality and mastitis control. These people include dairy producers, veterinarians, university researchers and extension specialists, milk procurement field staff, equipment and supply representatives, government officials, and students.

What can NMC do for you?

The continued pressure to ensure milk safety and improve milk quality, as well as the need to increase production efficiency, requires greater team effort between producers, veterinarians and other dairy professionals. Each team member plays a key role in developing successful mastitis control programs. NMC can serve as your resource for information related to udder health, milking management, milk quality, and milk safety.

Why join NMC?

- To receive the latest technical and applied information on udder health, milking management, and milk quality
- To provide leadership on milk quality issues within the industry
- To participate and learn about mastitis and milk quality developments at NMC meetings
- To establish valuable industry contacts
- To support education and research efforts that help raise awareness and understanding of milk quality issues

NMC membership benefits

- NMC annual meeting and regional meeting proceedings, containing all of the papers and posters presented at the meetings
- The NMC printed and electronic newsletters, addressing the latest information on udder health, milking management and milk quality
- Access to the "members-only" section of the NMC website, which includes the NMC Proceedings Library, NMC newsletter archives, and NMC membership directory
- Opportunities to network with other dairy professionals concerned with milk quality

*No other professional dairy
organization enjoys the wide
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Since 1961, NMC has coordinated research and education efforts to help control the losses associated with mastitis. By bringing together all segments of the industry, a strong and successful organization has been created to enhance the quality of milk and dairy products. NMC welcomes your active participation and support. Please visit the NMC website for additional information and resources.

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PAPERS

TEAT PREPARATION – WHAT ARE THE BENEFITS TO MILK QUALITY, UDDER HEALTH AND MILKING PERFORMANCE?

Morten Dam Rasmussen

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SUMMARY

Pre-milking teat preparation is not essential to a high milk production but do influence milk quality, udder health and milking performance. Pre-stripping is the most important step during manual teat preparation to detect clinical cases of mastitis, but the hand of the milker may also be a vector for spread of bacteria between quarters and cows. Milkers should wear gloves and cleaning procedures should leave the teat clean and dry at attachment. The method of cleaning teats at pre-milking teat preparation rather than the use of chemicals will affect the rate of new infections. In general, an efficient pre-milking teat preparation will lower the number of environmental cases of mastitis but may increase the number of cases caused by cow-dependent bacteria. An efficient and consistent milking routine will minimise milking on empty teats right after attachment, shorten the machine-on time and reduce the number of re-attachments for automated systems.

INTRODUCTION

It was good Latin for many years to stimulate cows well to obtain a full milk ejection and maximise milk yield. However, studies carried out for the last 20 years have not been able to show a milk yield benefit of good stimulation and most studies even without stimulation has shown no milk yield reductions in comparison with traditional manual or mechanical stimulation. We have heard pros and cons of the effect of teat preparation on milk yield and udder health so why not just drop it? Moreover, teat preparation may be a tedious procedure and especially for larger herds when milking takes several hours. There are several attempts to automate the milking process either fully as with automatic milking or parts of it. Milking in larger herds may take hours and it is an ergonomic challenge to do teat preparation consistently. Herds with full time pasturing often skip teat preparation and just apply the milking units as cows enter the parlour. This may work well during dry seasons, but it may not be practical all year around. This paper discusses the benefits and draw backs of teat preparation in relation to milk quality, udder health and milking performance.

MILK QUALITY

Pre-milking teat preparation consists of pre-stripping and of some sort of cleaning of the teats. Pre-stripping is mainly done to detect clinical mastitis as several countries legislate that abnormal milk must be withheld from delivery. Failure to pre-strip may seriously affect bulk milk cell count. Pre-stripping and withhold of abnormal milk from delivery is the single most efficient factor to reduce bulk milk cell count of problem herds. Besides, pre-stripping has only minor effects on the milk quality.

Inadequate cleaning of teat surfaces has in controlled experiments caused figures up to 20.000 cfu/ml milk (4). From a series of experiments conducted by Galton et al., (4, 5) it was concluded that some sort of wetting of the teat surface only followed by drying was needed to obtain low bacterial counts in milk, Table 1. Methods of udder preparation which wet the teat surface above teats cause drainage of bacteria-laden water onto teats if not properly dried. Washing of only dirty udders and teats was found to be no worse than washing of all udders and teats where teats subsequently drip-dried (11).

Table 1 Influence of pre-milking teat/udder preparation on bacterial counts in milk, cfu/ml (4, 5)

	Exp. 1	Exp. 2	Exp. 3
No udder preparation	17073a	6380a	11091a
Teats: dry towel	10654b	6117a	8012b
Udder and teats: water hose	19496a		
+ sanitizer	15398a		
+ drying	5547c		
Teats: water hose	5974c	6130a	
+ sanitizer	5632c	6196a	
+ drying	2116d	3259c	
Teats: wet towel	5033c	4467b	6314c
+ sanitizer	6547c	4695b	
+ drying	3763c	2045c	3555d
Teats: disinfectant dip		4203b	
+ drying		2938c	2976d

abcd: Means with different letters in same column differ (P<0.05).

Sanitizers in wash water were found to be of marginal or no benefit in reducing bacterial counts. Neither type of paper nor time spent on drying teats with paper towels influenced bacterial counts on teats (5). Cotton towels were found superior to paper towels in terms of reducing bacterial and spore count in milk and especially in procedures that include cleaning of the teat ends, Table 2.

Table 2 Effects of pre-milking teat preparation on standard plate count (SPC), spores of Clostridia, and residues of iodine in milk (17)

	SPC (ml)	Spores (L)	Iodine ($\mu\text{g/L}$)
No teat preparation	9317a	561a	
Dry paper towel for 6 s	3673b	257b	65ab
Wet and dry paper towels for 20 s	3005b	144cd	37ac
Cotton towel for 6 s	2232b	208bc	76ab
Cotton towel for 20 s	956c	118d	6d

abcd: Means with different letters in same column differ ($P < 0.05$).

Paper has a tendency to go smoothly over the dirt instead of removing it. The drawbacks of cotton are that it neutralizes halogens in wash water, especially at high temperatures, and certain bacteria can cling to the cotton yarns and thereby be protected. The use of cotton towels requires mechanical cleaning (washing machine) between milkings. Drying of teats after application of a pre-dip solution is mandatory. A cotton towel used for 20 s was found superior to other treatments in removing iodine from teats, Table 2.

Time spent on teat preparation is still an issue and new methods including pre-dipping and foaming products have been introduced. A study carried out in 8 herds in Southern England showed benefits of applying foam and wiping off before attachment of the milking units in comparison with dry wiping, wet methods, iodine predip and use of textile or disinfected towels (13). Use of the foaming products resulted in the lowest total bacterial counts of bulk milk, lower fecal strep count and a lower number of spores in milk. Two of the farms had high bulk milk counts during the period using foam for teat preparation. Differences in bacterial counts were not significant and other factors may have masked the results.

UDDER HEALTH

Dufour et al. (3) made a review on the effect of udder health management practices on herd somatic cell count (SCC) and found that the only significant pre-milking teat preparation method associated with low SCC was wearing of gloves. Fore-stripping, pre-milking teat disinfection and individual drying towels were not significant factors and could be present in low as well as in high SCC herds.

The hands of operators and towels are able to transfer pathogens from the teat surface of infected quarters to un-infected. Increased new infection rates have been shown where pre-stripping was combined with udder preparation when milking with a cluster which prevents cross-contamination (9). Transfer of pathogens occurred although pre-stripping was done before teat washing using rubber gloves and running warm water containing 500 ppm available chlorine and subsequent drying with a separate paper towel. By use of disinfectants in the wash water, the transfer

of pathogens is minimized only, not prevented totally. It is well established that post-milking teat dipping reduces new infections with cow dependent bacteria (15) and that the main target for pre-milking teat disinfectants is environmental bacteria, Table 3.

Table 3 New infections with *Str. uberis* in a challenge experiment (6)

	% cows	% quarters	% reduction
No preparation	62	27	0
Wet and dry paper	54	15	-43
Predip with 1000 ppm iodine and drying	25	9	-66

Pre-milking teat preparation and good udder hygiene reduce new infections with environmental bacteria and this shows that bacteria enter the teat canal during milking. Consequently, the number of bacteria at the teat end at attachment is a risk factor for new infections. Even though pre-milking teat disinfectants are bactericidal, they do not prevent all new cases of environmental infections. The two main factors to consider here are contact time and organic residues and farmers often fail to clean teats sufficiently before application and do not leave the pre-dip on for long enough time or even hit each teat with pre-dip. Hillerton et al. (10) conclude from farm studies that pre-dipping has no influence on udder health compared with general information about prevention of mastitis. Teats must be clean and dry before unit attachment in order to keep bacterial contamination of the milk as low as possible. Any procedure that wets the udder surface without proper drying afterwards will result in transport of bacteria towards the teat end (Figure 3), more bacteria drawn into in the cluster during milking, and reduced milk quality. In addition, milking wet teats has been shown to be associated with more clinical cases of *Streptococcus dysgalactiae* mastitis (1) and poorer udder health in general (14).

Attachment of the milking unit on empty teats causes vacuum to build up in the teat cistern following the pulsation and reverse pressure gradients across the teat canal may occur during these periods (19). Reverse pressure gradients may be the method of transferring bacteria through the teat canal during milking and this may explain why farmers applying a consistent milking routine experience fewer new infections (20). In conclusion, the method of cleaning teats at pre-milking teat preparation rather than the use of chemicals will affect the rate of new infections.

MILKING PERFORMANCE

The milking unit can be attached directly without teat preparation, but this procedure will especially for cows in later stage of lactation cause milking on empty teats. The amount of cisternal milk increases with the time between milkings and decreases as lactation progresses. Milking on empty teats of

peak lactation cows may be avoided if milk ejection occurs at attachment whereas late lactation cows must be stimulated at least 30 s prior to attachment. Bimodal milk flow curves were observed of 57-82% of not stimulated cows compared to 3% of stimulated cows (8, 21). Increased time spent of pre-milking teat preparation decreases the time until steady milk flow but effects are small compared to those of increasing the interval between pre-stimulation and attachment (18).

Start of milk ejection is a function of udder filling i.e. the amount of available cisternal milk in the udder at attachment. This percentage may vary from about 20% for cows in late stage of lactation up to 100% for high producing cows. There is a linear relationship between degree of udder filling and delay until milk flow at the start of milk ejection (2). For low percentages of udder filling milk is only stored in the alveoli and not immediately available for the milking machine. Kaskous and Bruckmaier (12) set up a couple of experiments to find the best combination of time spent on teat preparation and lag-time until attachment that would avoid bimodal milk flow. Pre-stimulation lasted from 15 to 45 s a lag times from 0 to 60 s. Milk yield, machine-on time and average milk flow was independent of the treatments if udder filling was >40%. For cows with an udder filling <40% increasing lag time decreased machine-on time independent of time spent on pre-stimulation. The authors conclude that a short pre-stimulation followed by a lag time up to 45 s is sufficient to start milk ejection and a suitable alternative for longer stimulation times.

The maximum benefit of pre-milking stimulation will be achieved if the milking unit is attached after teat preparation but allowing enough time for the milk ejection response to reach its peak. Consistent attachment about 60-90 s after the beginning of teat preparation can improve milk yield (16) and make the milking more efficient. First lactation cows, and especially in their early stage of lactation, have relatively small cisternal capacities which makes them more susceptible to poor milking and changed in preparation lag time. Older cows have relatively large cisternal capacities and in a large part of the lactation the cisterns will hold enough milk for the first half minute of milking until milk ejection is fully evoked, thereby avoiding milking of empty teats. Moreover, a consistent milking routine improves automatic detachment and reduce number of re-attachments. Ginsberg et al. (7) adjusted and optimized milking routines of 30 herds in Israel and had a special focus on milkers not to override the automatic take-off setting. Irregular take-offs were reduced from 62 to 3%, slow cows from 12 to 5% and machine-on time dropped from 5.9 to 4.9 min. This study shows the importance of applying teat preparation in a consistent manner in order to milk cows gently and quickly.

It takes longer time to milk out the last kg of milk if premilking teat preparation is conducted less efficiently or even omitted. Consequently, a good premilking teat preparation, a short, consistent interval until attachment, and calm cows are a prerequisite for detachments at high flow

rates. Cows will respond with short machine-on times, excellent teat condition, and proper milk out.

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NOTES

PCR VERSUS BACTERIOLOGY: DETECTION OF MASTITIS PATHOGENS BY REAL-TIME PCR IN CLINICAL AND SUBCLINICAL MASTITIS SAMPLES

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SUMMARY

Bacteriological culture methods are still the gold standard detecting bacterial pathogens in milk samples. Although, diagnostics of pathogens can be done by PCR. We compared the detection of mastitis pathogens by real-time multiplex PCR with conventional culture method using 104 clinical mastitis milk samples and quarter milk samples (n=100) from 25 cows with subclinical mastitis.

The average relative sensitivity of the real-time PCR for the detection of mastitis pathogens was 84.8% for clinical mastitis samples, and 93.8% for milk samples of cows with subclinical mastitis, when compared with bacteriological culture without pre-enrichment. Compared with culture results of pre-enriched milk samples, the average relative sensitivity of the real-time PCR was 81.7% for clinical mastitis samples, and 85.9% for quarter milk samples of cows with subclinical mastitis. Twenty (95%) of the 21 culture-negative clinical mastitis samples and 25 (66%) of the 38 culture negative quarter milk samples from cows with subclinical mastitis, were positive for mastitis pathogens by real-time PCR.

This study showed that the real-time multiplex PCR system evaluated detected more minor and major pathogens in both clinical and subclinical mastitis samples than bacteriological culture, even after pre-enrichment treatment of samples before culturing.

INTRODUCTION

The identification of mastitis pathogens is important in the management of mastitis. Culturing bacterial pathogens is considered to be the gold standard for diagnosis of pathogens involved in mastitis (5). However, culture and identification of bacteria is time-consuming and the sensitivity may be limited. Previous studies have reported that no causative bacterial agents could be found in approximately 30% of milk samples derived from clinical mastitis cases (1; 6). A recent study, using PCR techniques, showed that these 'no-growth samples' often contained mastitis pathogens in large

quantities, and it was speculated that the bacteria were either growth-inhibited or dead, thus providing false-negative results (10).

Although PCR has become an important tool in clinical and veterinary diagnostics, the number of reports on the use of PCR as a routine diagnostic tool in mastitis, is still limited (7; 2). Recently, a real-time multiplex PCR assay (PathoProof Mastitis PCR Assay®, Finnzymes, Espoo, Finland) was developed, which was described to have a high level of analytical accuracy in the identification of bacterial strains isolated from bovine mastitis samples (3).

In this study, the results of a real-time multiplex PCR were compared with culture results of mastitis samples from Dutch dairy cows.

MATERIALS & METHODS

Milk samples from clinical mastitis cases (n=104) were collected from 101 cows in 67 herds. Additionally, quarter milk samples (n=100) were collected from 25 cows in 5 herds, with elevated composite somatic cell count (SCC >250,000 cells/ml) but without clinical signs of mastitis.

Bacteriological culture

Bacteriological culture of the milk samples was done at the Animal Health Service. 10µl of milk were inoculated on blood agar and on Edwards plates, respectively, and incubated at 37°C. Plates were observed for bacterial growth after an incubation period for 24 and for 48 hours.

In order to increase the sensitivity of the bacteriological culture method, milk samples were cultured again after freezing the samples for at least 1 hour, followed by incubation of the sample at 37°C for 24 hours. This pre-enrichment step has been reported to enhance the growth of e.g. intracellular bacteria (9). Bacteria were identified by using standard biochemical assays as recommended by the NMC (5).

Real-time PCR assay

A volume of 350µL of milk (without pre-enrichment treatment) was used for DNA extraction and real-time PCR using the PathoProof Mastitis PCR Assay®, following the guidelines recommended by the manufacturer.

The assay identifies in four separate multiplex real-time PCR reactions a total of 11 mastitis-causing bacteria species or groups: *Staphylococcus* (*S.* *aureus*, *Staphylococcus* spp. (including *Staph. aureus* and all relevant coagulase-negative staphylococci (CNS)), *Enterococcus* spp. (including *E. faecalis* and *E. faecium*), *Corynebacterium* (*C.*) *bovis*, *Escherichia* (*E.*) *coli*, *Streptococcus* (*S.*) *dysgalactiae*, *S. agalactiae*, *S. uberis*, *Arcanobacterium* (*A.*

pyogenes/Peptoniphilus indolicus, Klebsiella spp., and the β -lactamase gene (*blaZ*) encoding for penicillin resistance in staphylococci.

Statistical analysis

For each pathogen the relative sensitivity of the real-time PCR was calculated separately by comparing results against the gold standard of bacterial culturing with and without pre-enrichment, by applying the following formula (4): Relative sensitivity % = (number of culture positive samples that were positive in the real-time PCR/number of culture positive samples) * 100.

RESULTS

Clinical mastitis samples, without pre-enrichment

The culture method detected in 83 (80%) samples minor and/or major mastitis pathogens, while 101 (97%) were positive by real-time PCR (Table 1). In 57 clinical mastitis samples (55%), real-time PCR detected more pathogens than the culture method. Bacteriological culture identified a total of 92 mastitis pathogens in the 104 clinical mastitis samples. Of these 92 mastitis pathogens, 78 were found by the real-time PCR, revealing an average relative sensitivity of 84.8%. The relative sensitivity of the real-time PCR for each of the mastitis pathogens ranged from 56% (*C. bovis*) to 100% (*S. dysgalactiae* and *S. uberis*) in the clinical mastitis milk samples without pre-enrichment.

In total 21 samples were culture-negative, but 20 of them (95%) were positive for mastitis pathogens by real-time PCR. In these 20 clinical mastitis samples, real-time PCR detected *E. coli* (n=2), *E. coli* + *C. bovis* (n=2), *E. coli* + CNS (n=4), *E. coli* + *C. bovis* + CNS (n=1), *E. coli* + *S. uberis* + *S. aureus* (n=1), *E. coli* + CNS + *S. uberis* (n=2), *S. uberis* (n=2), *S. uberis* + *E. coli* (n=1), *S. uberis* + *S. aureus* (n=1), *S. dysgalactiae* + CNS (n=1), *S. dysgalactiae* + *E. coli* + *A. pyogenes* (n=1) and *S. dysgalactiae* + *A. pyogenes* (n=1) and CNS + *S. uberis* + *C. bovis* (n=1).

Table 1 Results of bacteriological cultures with and without pre-enrichment compared with the PCR results of clinical mastitis samples.

	Milk samples of clinical mastitis cases (n= 104)	
	Without pre-enrichment	With pre-enrichment
Culture positive	80%	88%
PCR positive	97%	ND**
Number of pathogens found by culture	92	120
Number of pathogens found by both PCR and culture	78	98
Average relative sensitivity real-time PCR	84.8%	81.7%

*ND = not determined

Clinical mastitis samples, with pre-enrichment

In 91 (88%) of the 104 samples minor and/or major pathogens were detected by the bacteriological culture method after pre-enrichment (Table 1). In 42 clinical mastitis milk samples, real-time PCR detected more pathogens than bacteriological culture of pre-enriched samples. In total, the real-time PCR detected 107 minor or major pathogens which were not detected by bacteriological culture.

13 milk samples were culture-negative after pre-enrichment. In all of them one or more mastitis pathogens were detected by real-time PCR (Table 2 – see over).

Bacteriological culture identified a total of 120 mastitis pathogens in the 104 clinical mastitis samples after pre-enrichment. Of these 120 mastitis pathogens, 98 were found by the real-time PCR, revealing an average relative sensitivity of 81.7%. The relative sensitivity of the real-time PCR for each of the mastitis pathogens ranged from 60% (*C. bovis*) to 100% (*S. dysgalactiae* and *S. uberis*).

Staphylococcus spp. were detected more often by bacteriological culture than by real-time PCR (n= 13). In 7 of these 13 clinical milk samples *S. aureus* was only found after culture of pre-enriched milk samples.

Table 2 PCR results (Ct-values) of culture-negative (after pre-enrichment) milk samples from clinical mastitis cases (n=13).

Milk sample	Real-time PCR (Ct-value)
1	<i>E. coli</i> (20,3)
2	<i>A. pyogenes</i> (32,7); <i>S. dysgalactiae</i> (36,6)
3	<i>S. dysgalactiae</i> (35,9); <i>E. coli</i> (36,1); <i>A. pyogenes</i> (37,1)
4	<i>S. uberis</i> (26,6)
5	<i>E. coli</i> (32,9)
6	<i>E. coli</i> (23,9); <i>C. bovis</i> (33,3)
7	<i>S. dysgalactiae</i> (33,3); CNS <i>blaZ</i> neg (35,4)
8	<i>E. coli</i> (32,3); <i>C. bovis</i> (33,7); <i>S. dysgalactiae</i> (36,4)
9	<i>E. coli</i> (36,4)
10	CNS <i>blaZ</i> pos (29,8); <i>S. uberis</i> (34,1); <i>C. bovis</i> (36,4)
11	<i>E. coli</i> (32,5); CNS <i>blaZ</i> neg (35,4)
12	CNS <i>blaZ</i> neg (34,4); <i>E. coli</i> (34,5); <i>S. uberis</i> (36,2)
13	<i>S. uberis</i> (36,1); <i>E. coli</i> (36,5)

Quarter milk samples from cows with subclinical mastitis, without pre-enrichment

In 62% of the 100 quarter milk samples bacteria were cultured, while 86 samples were positive by real-time PCR. In 30% of the samples real-time PCR detected more mastitis pathogens than the bacteriological culture.

Using the bacteriological culture, 64 mastitis pathogens were detected and identified in the 100 quarter milk samples in total, of which 60 were found by the real-time PCR, revealing an average relative sensitivity of 93.8%.

The relative sensitivity of the real-time PCR for each mastitis pathogen ranged from 90% (CNS) to 100% (*S. uberis*).

Of 38 culture-negative milk samples without pre-enrichment, 25 (66%) were positive by real-time PCR. In these 25 samples real-time PCR detected CNS (n=11), *C. bovis* (n=11), *S. uberis* (n=1), CNS + *S. uberis* (n=1) and CNS + *S. aureus* (n=1).

Twelve of the 38 culture negative samples had a quarter SCC >250,000 cells/ml. In 10 (83%) of these 12 milk samples, mastitis pathogens were detected by real-time PCR: CNS (n=4), *C. bovis* (n=5) and CNS + *S. aureus* (n=1).

Quarter milk samples from cows with subclinical mastitis, with pre-enrichment step

Following pre-enrichment of quarter milk samples, bacteriological culture identified mastitis pathogens in 84% of the 100 quarter milk samples (Table 3). In 4 of the 16 culture-negative milk samples, the real-time PCR detected mastitis pathogens: *C. bovis* (n=3) and CNS (n=1).

Table 3 Results of bacteriological cultures with and without pre-enrichment compared with the PCR results of subclinical mastitis samples.

	Milk samples of subclinical mastitis cases (n= 100)	
	Without pre-enrichment	With pre-enrichment
Culture positive	62%	84%
PCR positive	86%	ND*
Number of pathogens found by culture	64	99
Number of pathogens found by both PCR and culture	60	85
Average relative sensitivity real-time PCR	93.8%	85.9%

*ND = not determined

In 18% of the 100 milk samples, real-time PCR detected more mastitis pathogens than bacteriological culture with pre-enrichment, and real-time PCR identified 41 mastitis pathogens which were not detected by the bacteriological culture method, even after pre-enrichment.

After pre-enrichment, bacteriological culture identified a total of 99 mastitis pathogens in 84 of the 100 milk samples. Of these 99 mastitis pathogens, 85 were found by the real-time PCR, revealing an average relative sensitivity of 85.9%. The relative sensitivity of the real-time PCR for each of the mastitis pathogens ranged from 79% to 100%.

Staphylococcus spp. were detected more often by bacteriological culture than by real-time PCR (n=11). In 10 of these 11 milk samples staphylococci were only found after culturing of pre-enriched milk samples. Additionally, 3 other subclinical milk samples that contained *Corynebacterium* spp. (n= 2) or *E. coli* (n= 1) were positive by bacterial culturing only after pre-enrichment.

DISCUSSION AND CONCLUSIONS

The results of this study show that the average relative sensitivity of a real-time PCR compared with bacteriological culture for the detection of minor and major pathogens was >80% for clinical mastitis samples and >85% for quarter milk samples from cows with subclinical mastitis. The average relative sensitivity of the real-time PCR slightly decreased when results were compared with the bacteriological culture results after pre-enrichment of milk samples. This reduction is probably due to the low levels of staphylococci in a number of the examined milk samples which were not detected by the PCR and only isolated by culture after pre-enrichment. This is in line with findings of Sol *et al.* (9), who reported that pre-enrichment of samples enhanced the growth and detection of mastitis pathogens.

On the other hand, in a large number of clinical and subclinical mastitis samples, the real-time PCR method detected more minor and major pathogens than were isolated by the bacteriological culture method. Minor and/or major pathogens were detected in 20 (without pre-enrichment) and 13 (with pre-enrichment) culture-negative clinical mastitis samples, and in 25 (without pre-enrichment) and in 4 (with pre-enrichment) culture-negative quarter milk samples from cows with subclinical mastitis. The results of the duplicate real-time PCR test run confirm the presence of bacterial DNA in the culture-negative milk samples.

One of the disadvantages of using PCR as a diagnostic tool is that it only detects the pre-determined bacterial species. For the real-time PCR system investigated in this study, bacteria such as *Bacillus* spp., *Pseudomonas aeruginosa* and yeasts will not be detected. In addition, this makes it more difficult to determine whether results are associated with sample contamination or not. To avoid misinterpretation of milk samples of poor quality, milk samples for real-time PCR need to be collected according to strict aseptic protocols. Detecting minor and major pathogens in culture negative milk samples by PCR may be due to lower detection limits, but may also be due to the presence of non-viable mastitis pathogens i.e. following antibiotic treatment or affected by the cow's immune response. Thus, the clinical impact of finding bacteria by real-time PCR still needs to be further studied.

This study shows that the evaluated real-time PCR detects more mastitis pathogens in clinical mastitis samples and in quarter milk samples from cows with subclinical mastitis than bacteriological culture does. Thus, the proportion of culture negative samples from clinical and subclinical mastitis cases will be reduced. However, more research is warranted to evaluate the clinical relevance of detecting more pathogens by real-time PCR.

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NOTES

THE ROLE OF MASTITIS VACCINES

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OVERVIEW

This paper summarises the recent vaccine research with respect to common causes of mastitis in the UK. It summarises the vaccines currently commercially available in the UK and possible vaccine regimes currently used on farms.

INTRODUCTION

When looking at the pathogenesis of mastitis as with any other infectious disease we need to consider the effect of the environment, specific pathogens and cow factors which would include the cow's ability to produce an innate immune response. As a result the control of mastitis is a multifactorial process which includes:

- Providing an appropriate environment (clean, dry, well ventilated)
- Milking machine working at its optimum with regular checking/servicing
- Good milking routine/hygiene
- Prompt identification, treatment and management of cases
- Effective pasture management/rotation
- Attention to detail
- Excellent staff/training

These last 2 points are difficult to quantify and rarely discussed in mastitis plans but are fundamentally important if change is to be implemented and progress is going to be made whether using vaccines or not.

Recent work in the area of mastitis has also illustrated the importance of the dry period as a risk for mastitis and this has been backed up by findings from the 800 or so farms that have been enrolled and evaluated as part of the Dairy Co Mastitis Plan (www.mastitiscontrolplan.co.uk).

The Dairy Co mastitis plan has been an important tool for veterinarians and other consultants working in the field of milk hygiene to address some of the risk factors involved with mastitis. It is interesting, however, that only one of the 377 questions deals with the use of vaccination on farms.

MASTITIS VACCINES

Effective mastitis vaccines have been a goal for researchers and pharmaceutical companies for many years. However the nature of the disease does not lend itself to a straight forward approach to vaccination. The principles behind vaccination tend to involve the enhancement of an immune response to a particular pathogen and as mastitis is defined as inflammation of the mammary gland there is potential for contradiction immediately.

Throughout the world the measurement of somatic cells in milk is used as a gauge by the dairy industry to milk quality and the absence (or indeed presence of infection). Part of the immune response that is triggered by a vaccine would include the migration of somatic cells, particularly neutrophils, to the mammary tissue. This may lead to milk of unsaleable quality in some circumstances.

Mastitis is a disease that can be potentially caused by a host of different pathogens (1, 2). Many of these are opportunistic pathogens. It is very possible that some pathogens will be protected against but in effect may be substituted by other pathogens leading to little difference in overall new intramammary infections (A. Bradley. Personal Communication).

Evaluating mastitis vaccines

How do we deem a vaccine to have been successful? At first sight we would like to see an elimination of mastitis infections within the herd. However this is not realistic. Average levels of mastitis in the UK are between 60 to 65 cases per 100 cows per year (1) with a proposed target (MAR) of 50 cases per 100 cows per year. However this is not as straight forward as this as there are many other parameters to measure and each producer/researcher will put a different emphasis on each one. Some of these include:

- Reduced Frequency of mastitis cases
- Reduced severity of mastitis case
- Reduction in Somatic cell count
- Increased spontaneous cure rates
- Increased cure rates following treatment in lactation and dry period
- Increased yield
- Less fatalities
- Reduction in classic 'toxic' cows
- Reduction in lost quarters

Before we start advising farmers to vaccinate against mastitis, are we confident that we have a true understanding of the epidemiology of the mastitis on that particular unit with particular reference to cure rates?

Without the analysis that is provided from software such as the Dairy Co Mastitis Plan (Sum It- QMMS) then this is impossible.

The nature of mastitis should also be taken into account. At the two different ends of the spectrum we have *E. coli* where the disease has traditionally expressed itself as one of a clinical manifestation and the associated problems while with *Staph. aureus* we tend to deal with a chronic disease that may be subclinical. *Strep. uberis* often sits in the middle of this spectrum behaving as either a contagious or an environmental pathogen and cure rates can often be a problem in a farm situation.

Innate immunity

While the use of vaccine may provoke an acquired immune response we may be better served in ensuring that the animal's innate immunity is providing the best possible protection. There is well documented evidence to suggest ways of maximising the natural immune response of an animal and this certainly should be maximised. The period around calving is one specific time when the immune system of the dairy cow may be compromised. Nutrition may play an important role at this time and a plethora of work has looked at the roles of Vitamin E, Selenium, Vitamin A, and Zinc to name but a few (3) as well as dry cow management. This is one area that may be worth investigating before going down an expensive vaccination route.

Vaccine licensing

It is important to note that there are different licensing rules across the world in order to bring a vaccine to market. For example in some countries it is only a prerequisite to show that a vaccine does not harm the animal nor has any contraindications. In Europe however, for an EMEA (European Medicines Agency) licence to be granted, clear efficacy needs to be demonstrated. For this reason in many parts of the world there are already a plethora of mastitis vaccines that are not used in Europe.

E. Coli vaccines

E. coli vaccines have been available in the UK for considerable time. These 'J5' vaccines produce antibodies directed against common core antigens that gram-negative bacteria share. These vaccines have been shown to be efficacious in US conditions (4,5,6) and anecdotally they have been seen to have a positive effect in UK conditions.

By exposing the core antigen, J5 vaccines enhance the ability of white blood cells to destroy bacteria. Vaccinated cows become infected with gram negative mastitis pathogens at the same rate as control animals but have a lower rate of development of clinical mastitis (4). Furthermore the use of the vaccine reduces the duration of clinical infection (6).

Staph. aureus vaccines

Commercially available vaccines for *Staph. aureus* in the United States have been shown to have limited ability to prevent new infections. (7,8). However vaccine has been shown to have the effect of enhancing the spontaneous cure rates of vaccinated cows (9,10). The other main effect seems to be a reduction in the number of chronically infected subclinical cows that go onto become clinically infected (11). The approaches to *Staph. aureus* vaccination tends to be aimed at virulence factors. These have included:

- Proteins in bacterial cell walls (Protein A)
- Adhesion factors on bacterial cell walls
- Slime pseudocapsules

There is only one commercially available vaccine available for *Staph. aureus* in the UK at present. The vaccine contains Slime Associated Antigenic Complex (SAAC). The antibodies produced adhere to the biofilm and aid opsonisation by neutrophils. The biofilm in this case is common to different strains of Staphylococci, including coagulase negative staphylococci.

The trial work associated with this vaccine has been mostly carried out in Spain under different conditions to those experienced to the UK. The percentage of cows with a SCC greater than 200,000 in the first month after calving was reduced by 19.8% in the vaccinated group (12).

Results of other field studies indicate that the vaccine is efficacious in the reduction of the incidence of intramammary infection due to coagulase-negative staphylococci, with clinical or subclinical manifestations. Immunization also improve the spontaneous cure rate of CNS IMI, the number of mastitis treatments required and diminish the percentage of cows with significant decrease in the expected milk yield (13).

Early anecdotal evidence from further studies in Europe suggests that the vaccine is helping to reduce cell counts with high numbers of chronically infected cows. The mechanism for this has yet to be elucidated but it is postulated that the vaccine is having an effect on breaking down the established slime layer (R. Guix, HIPRA, Personal communication).

A large scale trial is currently being performed using this vaccine in UK conditions. The results are eagerly anticipated. (The vaccine in question also contains a J5 component).

Workers in Israel have been researching a recombinant TRAP (Target of RNAlII activating protein) vaccine. This TRAP is a membrane associated protein. Studies have shown a humoral response lasting for 160 days followed by a repeat vaccination in mid lactation. This has resulted in a decrease in cell count and increase in yield (14).

Strep. uberis vaccines

Strep. uberis is one of the most common causes of increased cell count and clinical mastitis (1,2). A vaccine against the organism would therefore be a major breakthrough in the control of mastitis. An in depth paper was presented at the 2010 British Mastitis Conference (21).

The issue of cross protection has been a major stumbling block in the production of an effective *Strep. uberis* vaccine in the past. Both live (15) and killed bacterin (16) vaccines have been trialled in the past but the strain specific protection that a live vaccine produced limited their application (17). Since the genomic mapping of *Strep. uberis*, attempts have been made to experimentally mutate the genome to reduce the ability of the organism to cause mastitis. These have included the hyaluronic acid capsule (18) and plasminogen activator (Pau A) (19). In both cases the virulence of the mutant strains was not reduced.

Manganese requires a special carrier (MtuA) to allow uptake into mammary cells and this was considered a possible area for vaccine development. However this carrier is not present on the surface of the cell wall and therefore is not accessible to the antibodies produced by a vaccine (20).

The University of Nottingham has concentrated its efforts on looking at the sortase enzymes (SrtA). These are 10 proteins responsible for anchoring proteins to the cell wall. Mutant strains of *Strep. uberis* however that did not express the sortase anchoring gene were less virulent. The research has now highlighted three of these proteins that were involved with the disease process. Of the three, the protein that is encoded by the gene sub1154, has shown the greatest potential. This protein is a protease similar to those encoded by other streptococci that degrade proteins in the innate immune response (17).

In his 2010 BMC review (21), Leigh also discusses recent research that suggests that there are strains of *Strep. uberis* present in the gut that are avirulent with respect to the mammary gland (22). Such strains could be used in a 'probiotic way' to prevent infection with other virulent strands. He also highlights that work to date has concentrated on reducing colonisation of the mammary gland but an alternative approach may be to look at controlling the life cycle of *Strep. uberis* in other areas including the gastrointestinal tract.

An alternative vaccine is being trialled at the University of Tennessee. *Strep. uberis* adhesion molecule is a surface molecule that binds lactoferrin thus enabling bacterial penetration of the host cell (23) Vaccination of dairy cows with a recombinant *Strep. uberis* adhesion molecule induces antibodies that reduce adherence to and internalisation of *Strep. uberis* into the bovine mammary epithelial cells (24).

No commercial vaccines for *Strep. uberis* are currently available in the UK.

COST BENEFIT

Cost benefit models for mastitis vaccination are often used to demonstrate the benefits to a farmer. These cost benefits at first glance seem easy to equate. This is certainly the case when looking at the use of J5 vaccines that tend to reduce clinical mastitis. However the effect of *Staph. aureus* vaccines is not so easy to evaluate. Big assumptions are made within the models. In the case of J5 the model assumes a 10% reduction in yield for every case of mastitis and a vaccine efficacy of 80%. However these do not take into account the potential reduction in treatment time or a reduced severity of mastitis. Cost benefit analyses need to be treated with care.

FARM CASE STUDIES AND VACCINATION PROTOCOLS

There is currently one commercially available mastitis vaccine in the UK (Startvacc, Hipra UK). This is licensed to be used for coliform mastitis and also for staphylococcal mastitis (including CNS).

The vaccine is designed to maximise immunity at and around calving with a three dose course to give protection for 130 days into lactation. It is important to monitor the patterns of mastitis across lactation to assess at what stage (Days in milk) that mastitis is occurring. Coupled with regular bacteriology this will enable the vaccination program to be reassessed. In many herds, lactational analysis and bacteriology have demonstrated coliform mastitis being a problem in later lactation. This had led to an off label usage of the vaccine.

Vaccine is given every 3 months to all milking cows to provide lactation long protection. Special care needs to be given to ensure that heifers receive vaccination at an appropriate time. It is therefore important to do a detailed analysis of heifer mastitis rates across the first lactation. In some instances it is more appropriate (and manageable) to vaccinate heifers for the first time after they have entered the herd. In other cases heifers need to enter the herd fully protected to maximise efficacy of the vaccine. As with many infectious diseases, heifer vaccinations can lead to major vaccine compliance issues and the author finds standard operating procedures and calendar reminders a vital tool for managing vaccination protocols in large herds. Bacteriology of both clinical cases and sub clinical cases is vital to build up a true picture of the disease situation on the farm. It is important to reiterate that the vaccine currently available offers no protection whatsoever against *Strep. uberis*.

In the author's experience the vaccine has been used principally to combat clinical mastitis rather than sub clinical mastitis and this is probably a continuation from the indication used for the previous J5 vaccine that the subsequent vaccine has replaced. It is important to note that in those herds

where vaccination has become part of the overall mastitis management plan that there are many other factors that are also in place to help tackle the mastitis situation (25). Anecdotally in the author's experience the main factors that are common to all these farms are:

- Attention to detail
- Quality staff
- Good communication
- Standard operating procedures
- Staff training
- Sand bedding
- Dairy Co Mastitis Plan in place

Vaccination for mastitis is still a relatively expensive procedure and a full cost benefit analysis is necessary. However a reduction of 1.5 cases per month in a 200 cow herd is required to justify the cost. It is important that it is not considered as a quick fix and maximising the cow's own innate immunity is the best way to reduce mastitis.

SUMMARY

Vaccines may have a role to play as part of an overall mastitis control plan. However a full investigation into the epidemiology of the mastitis situation on a particular unit must be carried out before vaccine is considered.

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NOTES

INFLUENCE OF GENETICS ON MASTITIS

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INTRODUCTION

Despite the widespread implementation of control programs mastitis continues to be one of the most economically significant diseases affecting the dairy industry and it is logical to assume that producers would seek to alleviate losses using a variety of methods including genetic selection.

There is a clear advantage in breeding cows with greater resistance to mastitis assuming that other production traits are not impaired. Benefits expected to accrue from such a strategy would include improvements in animal welfare and milk quality, reduced production losses both from discarded milk and damaged mammary tissue, economies in the use of veterinary therapeutics and services and the labour required for the care of sick cows. Opportunities for genetic progress in mastitis resistance however remain limited as mastitis is a complex disease involving many pathogens and many different defence mechanisms. It is likely that there are many hundreds of genes involved in mastitis resistance, which makes genetic improvement challenging if one is seeking the single gene or genes that will impart mastitis resistance. More realistic is the possibility of ongoing genetic selection for mastitis resilience rather than resistance which can be more easily measured using traits such as somatic cell count (SCC) or somatic cell count score (SCS).

The purpose of this paper is to examine the role of genetics in the control of mastitis and highlight some of the obstacles and progress made in our understanding of the influence of genetics on mastitis.

MASTITIS RESISTANCE

Considerable variation exists between individuals in the capability of cows to recover or resist intra-mammary infection [1, 28]. Very little difference however was found between breeds when Jersey and Holstein cows were challenged with *E. coli* or *S. aureus* [2, 3]. Some studies [28] have lacked the data from the early post infusion period and considering that early detection and rapid response are of paramount importance to the outcome of infection this is disappointing. It is clear however that some cows are inherently more resistant to mastitis than others.

For mastitis to occur bacteria must first penetrate the barrier to infection afforded by the teat and thus the conformation of the udder and teats is directly associated with the development of mastitis and has a moderate correlation (0.26-0.52) with clinical mastitis [25, 27]. Traits such as low

hanging udders, wide set teats and flattened teat ends have been associated with increased SCS and impaired mastitis resistance as they predispose the udder to exposure with pathogens [22, 26, 27], whilst long teat ends and increasing teat diameters contribute to mastitis via their association with liner slips during milking [26]. The moderate heritability values for udder conformation traits when compared to those of clinical mastitis and SCC should allow for more rapid genetic improvement in udder traits and so potentially a more rapid improvement in mastitis resistance.

Once bacteria have penetrated the gland a rapid rise in somatic cells, mainly neutrophils is one of the earliest responses of the host defences and it is largely the efficiency of this response which determines the duration of an infection. SCC or the logarithmic transformation of SCC, SCS is thus strongly correlated with clinical mastitis in the range 0.60-0.80 [5, 34] and is a useful tool in the selection process for mastitis resistance.

Neutrophil migration and function are essential for resolving bacterial infection [21] but to mobilise this response requires a complex network of pathogen detection and signalling pathways involving many genes which ultimately leads to the secretion of a multitude of inflammatory mediators [9] which include cytokines such as interleukin-8 (IL-8). The major role of IL-8 is to recruit neutrophils to the site of infection where they can engulf and kill an invading pathogen. The secretion of this single mediator alone is likely to involve many genes with many polymorphisms so ample opportunity exists for genetic or other environmental influences to result in between cow differences in mastitis susceptibility.

ACCURACY OF SELECTION

Traditional Phenotypic Selection

A major limitation in the progress of selection for mastitis resistance is the reliance on selection strategies based mainly on the measurement of SCC or its derivative SCS as a selection tool and while progress has been made in reducing SCC the actual effect on the incidence of mastitis either clinical or sub-clinical is difficult to measure as the correlation of SCS with clinical disease is approximately 0.70 and the phenotype of 'mastitis resistance' is ill defined and confounded by many factors such as pathogen presence and load, housing, milking routines and other management practices such that a cow that never experiences mastitis on one farm may not be so resistant when presented with an entirely different set of environmental circumstances.

Selection could be substantially improved if a genetic evaluation was available for clinical mastitis. Selection indices based on clinical mastitis are problematical for a number of reasons not least of which is that clinical mastitis is only one form of the disease. Sub-clinical disease is more prevalent but is difficult to evaluate as it requires more extensive monitoring

such as frequent culturing to determine its presence. This may change with the availability of molecular techniques such as real time polymerase chain reaction (RT-PCR).

Perhaps a greater issue is the level and accuracy with which clinical mastitis is reported in the field which varies considerably depending on the individual on farm definition. However studies have shown that in well managed herds on farm records are a feasible means of incorporating more information into genetic models [39]. Using data of this type the heritability of clinical mastitis has been estimated to be 0.10 and despite the low heritability of clinical disease and the reliance on indirect traits such as SCC or SCS progress in selection and breeding for disease resistance has been made particularly in the Scandinavian countries [6]. The use of SCS in combination with clinical mastitis records is more effective in predicting mastitis susceptibility than SCS alone in selection indices [20]

The level and accuracy of clinical disease reporting in many countries however remains low and is a major impediment to the development of accurate genetic indices and selection tools. A possible explanation for the lack of accurate reporting may lie in the distrust with which producers view official bodies and the suspicion that the information reported may be used to inflict a greater burden of regulation and cost onto the industry.

Genetic Marker Assisted Selection

Identification of genetic markers associated with mastitis that display Mendelian inheritance patterns would add greatly to mastitis selection programs. Two possible approaches can be taken to the search for genetic markers. Firstly positional identification searches for a region of the genome that is highly associated with the trait of interest, this region is more commonly known as the quantitative trait loci (QTL). Alternatively functional identification seeks to identify genes known to be important to the trait of interest which is then evaluated for the presence of single nucleotide polymorphisms (SNP) or single base pair changes that can be used as a marker. The individual SNP is then evaluated for its association with disease which may be directly causal or a link in a broader cascade of events.

Multiple QTL for both SCC/SCS and clinical mastitis have been located on bovine chromosomes [8, 12, 29,] suggesting that within or near these chromosomal regions are the genes that act to affect disease resistance. Further refinement of each QTL into smaller regions allows the search for the individual genes to be narrowed. The release of the bovine genome however makes it possible to identify genes within regions and directly search for SNP's that contribute directly to individual animal variation in disease resistance.

Health and fitness traits such as mastitis resistance and SCC are excellent candidates for marker assisted selection despite their low heritability's due to the large variation between individuals but as with traditional phenotypic

selection success will be limited by our ability to collate meaningful data on clinical mastitis events.

The major impediment then to providing effective genetic solutions to enhance mastitis resistance is the ability to accurately define a mastitis resistant phenotype [30]. Current phenotypic data relies heavily on the periodic measurement of somatic cell count which suffers due to missed cases of mastitis due to infrequent sampling and is based on composite samples taken from all four quarters resulting in dilution of the SCC from an infected gland.

The genetic marker assisted approach to selection has identified the CXCR2 gene as a potential marker for disease resistance. This gene is critically tied to the ability of neutrophils to survive, migrate, engulf and kill pathogens all of which are essential for the resolution of bacterial infections. Five SNP's have been identified in the CXCR2 gene of Holstein and Jersey cattle and of these 3 have been associated with sub-clinical mastitis in a small population of Holstein dairy cows [38]. The SNP located at position +777 has attracted research interest because a change in this nucleotide resulted in a single amino acid change in a G-protein signalling receptor which has the potential to affect disease resistance by affecting neutrophil function [37]. Neutrophils migrating from the blood to the site of infection must first adhere to the blood vessel lining requiring an increase in the expression of adhesion factors such as CD11b and CD18. Preliminary studies on small numbers of animals have shown that the up regulation of these factors was reduced in cows with a CC genotype relative to a GG genotype [24]. Neutrophil migration was also reduced in these studies which also indicated that cows with the GG or GC genotype may be more resistant to infection than cows of the CC genotype although the duration of infection once established was unaffected.

Tissue Model Assisted Selection

Recently skin fibroblasts have been used as a model for predicting the variation in cow response to mastitis [11]. The secretion of interleukin-8 (IL-8) into media following challenge with a variety of mastitis pathogens, toxins and cytokines is measured. This technique is attractive as these cells are readily available and can be grown under simple culture conditions following cryopreservation and transport but most importantly secrete large amounts of IL-8 in response to challenge with *E. coli* endotoxin with minimal basal secretion under control conditions. Current research programs are aimed at showing that variation in fibroblast response relates to variation in disease resistance and that the status of an animal and magnitude of response will not vary with age. This approach is clearly of interest as the capability to more accurately identify a disease resistant phenotype would greatly accelerate the rate of genetic progress as well as facilitating the genomic search for specific genes that confer superior disease resistance.

GENETIC REGULATION OF THE IMMUNE SYSTEM

The immune system comprises a highly integrated and regulated set of cellular and molecular responses to both internal and external stimuli including pathogenic micro-organisms [4, 18] and there is clear evidence that in many species including cattle it is possible to select for immune responsiveness [10]. Heritability estimates for both antibody and cell mediated immune responses are sufficient to allow for improvement to be made via genetic selection [17] and that these responses have an impact in reducing the odds ratio disease scores for mastitis as well as other diseases such as ketosis, metritis and retained placenta [16].

Recently High Immune Response (HIR) Technology [16] has begun to be applied to identify and select individuals with higher breeding values for immune response traits. A bovine immune-endocrine microarray chip has allowed the genes associated with individuals classified as high (H), average (A) or low (L) immune responsiveness to be identified [19, 35]. Certain bovine immune-responsiveness genes have also been shown to express single SNP's that correlate with high and low SCC alongside other dairy traits [14, 15, 22, and 33]. It remains to be seen if these SNP's also associate with H and L immune responsiveness.

Since both antibody and cellular immunity are crucial aspects of the hosts response to pathogens it is desirable to select animals with both high antibody and high cell mediated immune responses with the aim of producing animals with a balanced immune response capable of resisting a diverse variety of pathogens and it is interesting to note that animals can be further classified on the basis of their capability to produce either an antibody mediated immune response or a cell mediated response and that the response is at least in part influenced by both age and pregnancy [7]. It is noted however that a calf identified as a high immune responder will generally maintain that classification as a mature cow.

Epigenetics describes heritable changes in phenotype and gene expression which may last for the life of a cell or for many generations which are caused by mechanisms other than changes in the underlying genome. Examples of epigenetic changes would include DNA methylation and histone acetylation both of which serve to alter gene expression without altering the underlying gene sequence. Cellular differentiation in the embryo in which a single cell divides and becomes many cell types is an example of epigenetic regulation. Epigenetic effects on immune regulation in cattle have also recently been identified in the changes in immune response that occur during pregnancy and parturition [16]. In this instance changes in DNA methylation between bovine type 1 (interferon γ (IFN- γ)) and type 2 (interleukin 4 (IL-4)) cytokine promoter genes between 4 weeks prior to and 4 days post calving were identified. These two cytokines have opposing regulatory functions and the methylation patterns observed between these two cytokines are consistent with those reported for other species [36]. Epigenetics is now generally

thought to represent the vital connection between gene expression and the environment [23] and its influence on bovine cytokine genes that are known to steer the immune response can then be expected to play a crucial role on the nature of the immune response around the time of calving.

BREEDING COMPANY PERSPECTIVE

The transition in genetics companies breeding programs has been ongoing for some time as selection has switched focus from the traditional traits of production and conformation to place a greater emphasis on health and fitness traits. Amongst the underlying reasons for the increasing demand for improvements in health and fitness traits is undoubtedly the trend towards bigger herd sizes, which has also led to a rapid growth in the use of computerised mating programs and whilst the key to a breeding company's success lies in marketing the genetics that producers like and want the capability to avoid individual mating' that are likely to result in offspring that are extremely poor for a particular trait is also desirable and whilst our current evaluations for health and fitness traits may not be adequate enough to make a rapid improvement in the population the information can almost certainly be used to avoid mating' with undesirable outcomes.

CONCLUSIONS

High levels of production and expanding herd sizes emphasis the need for selection based on health and fitness traits and developing genetic evaluations for clinical mastitis is key to improving the success achieved with SCS evaluations but there are serious issues with data reporting which will need to be addressed. The progress currently being made in outlining regions and specific genetic markers coupled with new technologies to identify individuals with high immune responsiveness make it possible to enhance the genetic selection of cattle with greater resistance to mastitis and other diseases. However it is unlikely that there will be a single solution to the problem of disease resistance. Mastitis in particular is a complex disease involving many factors and it is likely that multiple strategies will be necessary to improve our ability to select for mastitis resistance.

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NOTES

MASTITIS AND INFERTILITY: PRELIMINARY RESULTS OF A LARGE RETROSPECTIVE STUDY

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SUMMARY

There is a well-established relationship between udder health and reproductive performance, but existing work in the field has a number of shortcomings and little recent work has been conducted in UK dairy herds. Preliminary results are presented from a study which aimed to improve understanding of this relationship by using a large retrospective dataset from UK dairy herds to construct a multilevel discrete time survival model, with probability of pregnancy being established during a 2-day risk period as the outcome variable and a number of udder health related potential explanatory variables.

INTRODUCTION

It has been recognised for some time that both clinical and subclinical mastitis can be associated with decreased reproductive performance in dairy cows. Much early research in the field took a similar approach to Barker *et al.* [1], whereby reproductive performance in a cohort of cattle which developed clinical or subclinical mastitis was compared to a control cohort [2,3]. Whilst this early work often demonstrated significantly impaired reproductive performance in affected cows compared to unaffected controls, this approach allows limited ability to account for other variables potentially confounding the apparent relationship between fertility and udder health. Subsequent work in the area has used regression modelling to evaluate the association between fertility outcomes and udder health status whilst accounting for the effects of other variables [4–7].

Recently, Hertl and co-workers [8] used this method to evaluate associations between clinical mastitis (CM) at different timings relative to an insemination and the outcome of the insemination, finding that CM between 14d before and 35d after a serve was associated with a decreased probability of a resulting pregnancy. In another recent study, Lavon *et al.* [9] found associations between intramammary infection status (as determined by individual cow somatic cell count history) and a significant reduction in pregnancy rate to first insemination.

There is therefore a large body of work suggesting that udder health and fertility performance are related, but there are a number of limitations of the existing knowledge:

- None of the more recent studies have used both CM and somatic cell count (SCC) as potential explanatory variables. This makes it difficult to understand whether one or other of these is responsible for the observed effects, or whether both CM and subclinical mastitis can depress fertility independently.
- Studies using regression modelling (i.e. those accounting fully for potential confounding variables) have tended to focus on the association between udder health and pregnancy rate (i.e. the probability of a serve leading to a pregnancy). This ignores the possible effects of udder health on submission rate (the probability of a cow being served in a given period of time), which some earlier studies using a cohort comparison approach had found to be significant.
- The majority of studies were conducted using a small number of herds, often on high-yielding units in the USA; the appropriateness of extrapolation of these results to the UK situation is not clear.
- Only the most recent studies [8,9] evaluated the importance of timing of udder health events in a sophisticated way.

This study was carried out in order to address these issues and improve understanding of this area.

MATERIALS & METHODS

A convenience sample was taken of 80 dairy herds from England and Wales which were considered by herd advisors to have a good standard of data recording. Routine management data was collected from these herds in electronic format (either from on-farm or bureau recording software, or from milk recording organisations) covering the years 1999 – 2008. Herd-level datasets were anonymised and converted into a consistent format, and auditing was performed to eliminate datasets which appeared to have missing calving, service, CM or SCC events. Dataset quality was evaluated at herd-year level, such that herds could contribute data for all or part of the 10 year period (as many herds had good quality data for only part of the period).

Data restructuring was then carried out so that each lactation was broken down into 2-day risk periods between 20 and 220 days in milk (DIM). A binary outcome variable representing whether or not a pregnancy was established in each risk period was calculated using the date of the subsequent calving. Risk periods after pregnancy had been established or after the cow was culled, sold or died were removed from the data. A number of potential explanatory variables were also calculated, some at lactation level (so that the value of the variable was the same for every risk period during a lactation) and some at risk period level. Variables related to udder health included binary representations of occurrence of CM at various timings relative to the risk period (covering the range from 70 days before to 90 days after the risk period) and categorical representations of individual

cow SCC (ICSCC) at various timings from 90 days before to 30 days after the risk period. In addition to these, a variety of other potential explanatory variables not directly related to udder health were calculated: these included DIM and season at risk period level, and 305 day milk yield, parity and year at lactation level. The final dataset consisted of 2,338,025 risk periods from 39,590 lactations in 21,068 cows.

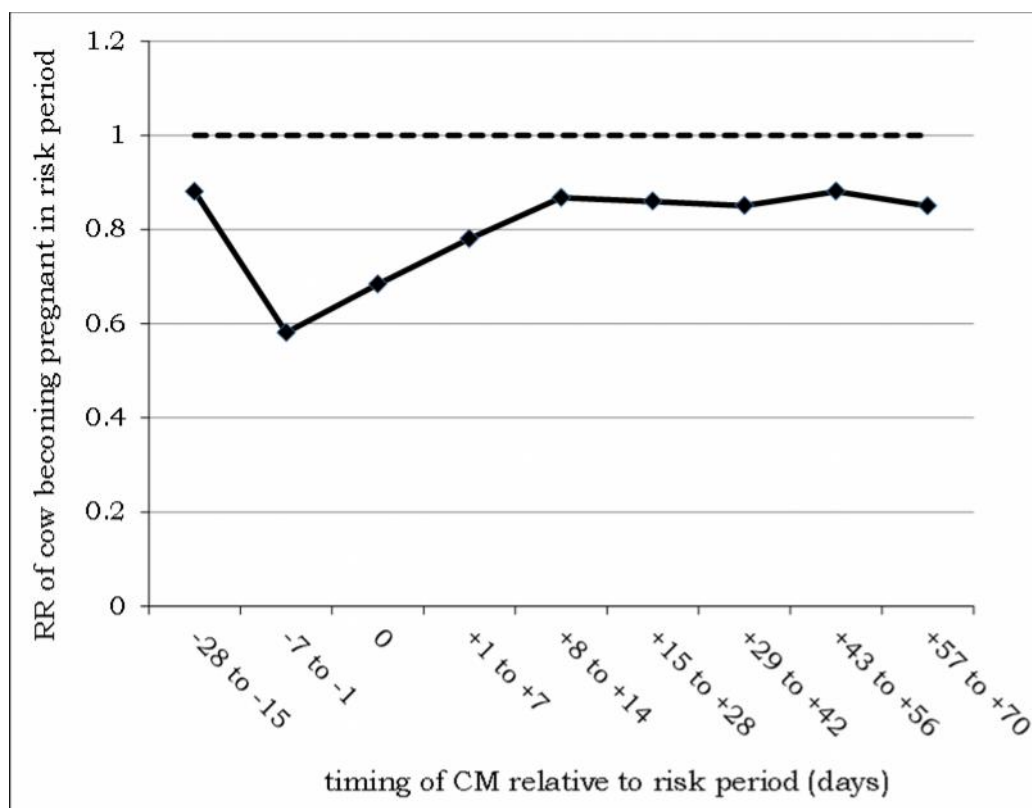
A discrete time survival model was then constructed [10], using the outcome of pregnancy becoming established during a 2-day risk period and the potential explanatory variables described above. A three level hierarchical structure was used (with risk periods nested within cows nested within herds), with random effects terms at cow and herd level (to account for any unmeasured variation between individual cows and between different herds). Model building was performed using forward selection in MLwiN version 2.20 [11], with Markov chain Monte Carlo used for final parameter estimation. Variables were retained in the model if the 95% credible interval for the estimate of the variable's coefficient did not include zero for at least one category of the variable. This process effectively uses the data to derive a best-fit equation allowing prediction of the probability of pregnancy occurring in a given 2-day risk period based on the explanatory variables. This allows evaluation of the effect of each individual explanatory variable *whilst accounting for* the effect of all the other explanatory variables, as well as individual variation between cows and between herds.

Simulation-based posterior predictions (run over 10,000 iterations using a subset of 100,000 risk periods from the main dataset) were generated using WinBUGS version 1.4 [12] to check model fit and to present results as relative risks (RRs). Model predictions were close to observed values, suggesting that model fit was good and therefore that the model was an appropriate way to represent the data. Predicted RRs were calculated as an alternative method of presenting model results (conventionally, odds ratios would be used for this type of analysis): risk is an intuitively easier concept to deal with than odds, so relative risks provide a simpler way to interpret results.

RESULTS

The overall mean probability of a pregnancy being established during a 2-day risk period was 0.0125: this would correspond to just over 13% of cows becoming pregnant every 21 days. Preliminary results showing associations between CM history and relative risk of pregnancy during a 2-day risk period are shown in Figure 1.

Figure 1 Predicted relative risk (RR) of pregnancy during a 2-day risk period for cows with a case of clinical mastitis at various timings relative to the risk period, compared to a cow with no clinical mastitis (dashed line).



It is clear that CM close to the risk period is associated with a major reduction in probability of a pregnancy occurring. The largest effect size occurred where a case of CM was recorded 1 to 7 days before the risk period (RR \approx 0.6); CM during the risk period was associated with a decrease of around one third in the risk of pregnancy (RR \approx 0.68) and CM 1 to 7 days after the risk period associated with a smaller reduction in risk again. A case of CM occurring between 8 and 70 days after the risk period was associated with a reduction in risk of pregnancy of around one tenth (RRs \approx 0.9), and a case of CM 15 to 28 days before the risk period had a similar association. It is important to emphasise that these RRs represent the predicted effect of CM where the other explanatory variables are held constant (so there would be an additive effect where, for example, CM was associated with an elevation in ICSCC, or where multiple cases of CM occurred within a single lactation).

DISCUSSION

Preliminary results from this study support the findings of early workers in this field, suggesting that CM is associated with a decrease in overall reproductive performance. However, this study may be the first to

demonstrate an apparent effect on overall reproductive performance (as opposed to pregnancy rate) whilst taking full account of potential confounding factors.

Early indications suggest that CM is associated with decreased fertility over a prolonged range of time, from 28 days before to 70 days after a risk period, with the largest apparent effect seen in the week before the risk period. This is different to the order of effect sizes reported by Hertl *et al.* [8], who found that the greatest effect on pregnancy rate occurred when CM was recorded around or just after an insemination. It is plausible that this difference is due to the effect of CM just before oestrus on the probability of a cow being served (which would not have affected the outcome of a study using pregnancy rate as an outcome). This suggests that CM just before a risk period had a substantial negative effect on the likelihood of the cow being served during the risk period: this could either be related to management decisions not to serve where an oestrus was observed shortly after a case of CM, or to a suppressive effect of CM on ovulation or expression of oestrus.

Although this study may provide evidence regarding the possible effect of udder health on reproductive performance at lactation level, the importance of this at herd level is less clear. Of particular potential significance here is the early finding that the total effect of CM is greater where it occurs after a risk period; the apparent effect of CM more than 7 days before a risk period was small. This is important as the majority of CM cases tend to occur in early lactation. A substantial proportion of cases of CM may therefore have little effect on lactation reproductive performance as they occur before cows are eligible to be served, especially in herds where a large proportion of CM is of dry period origin. Further work is currently in progress to explore the relative importance of udder health compared to other influences on overall reproductive performance.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of SUM-IT computer systems in this work.

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NOTES

ANTIMICROBIALS – PAST, PRESENT AND FUTURE

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SUMMARY

In the past five years there has been a lot of discussion regarding the use of antibiotics in animals at both a national and European level. This paper considers the use of antimicrobials in animals in Europe and the UK. There are potential risks to the use of antibiotics in animal medicine as the concern around antibiotic resistance grows. There is a need for safe efficacious antibiotics in both human and veterinary medicine, to ensure the health and welfare of both humans and animals. All sectors of the agricultural industry need to be involved in any discussions around the use or possible restrictions on use in animals that may be imposed.

ANTIBIOTICS

Before the early 20th century, treatments for infections were based primarily on folklore. Mixtures with antimicrobial properties that were used in treatments of infections were described over 2000 years ago. Many ancient cultures, including the Egyptians and Greeks, used specially selected mold and plant materials and extracts to treat infections. More recent observations made in the laboratory of antibiosis between micro-organisms led to the discovery of natural antibacterials produced by microorganisms. In the late 1880s, in Germany, synthetic antibiotic chemotherapy and development of antibacterials began. Penicillin, in 1928, was the first antibiotic discovered and is still commonly used to treat infections today.

Antibiotic use is essential in both human and veterinary medicine. However we need to use these medicines wisely and prudently or these miracle drugs of the 20th century will no longer be available for the animal industry either through politics or reduction in efficacy.

Human Medicine

Antibiotics are amongst the most frequently prescribed medicines in modern medicine. More and more people are self diagnosing and there is greater availability of information and medications through the internet. Misuse and overuse of antibiotics is the most important cause of antibiotic resistance.

In a very worrying survey that was carried out, in the US in 1998 (1), it was found that 52% of individuals said they believe antibiotics are the best medicine for viral infections. Physicians, who know that antibiotics are not effective against viruses, often go along with their patients' desires. Surveys of paediatricians reveal that they prescribe antibiotics to 44% of patients

with viral infections such as common colds, 46% with upper respiratory tract infections, and 75% with bronchitis. Physicians treating adults with viral infections prescribed antibiotics for 51% of patients with colds. (1)

Efficacious antibiotics are critical in human medicine to treat disease and to ensure human welfare. Prior to the discovery of antibiotics millions of people died around the world from what we would now see as easily treatable infections. Unfortunately it is the most vulnerable of people that suffer the most from antimicrobial resistance, i.e. the young, the old and the immune-compromised.

Veterinary Medicine

Where possible vaccination and management control measures should be used to prevent disease. However in certain circumstances antibiotics are needed to treat and control disease in animals. The availability of antibiotics to treat animals is critical to animal health and welfare.

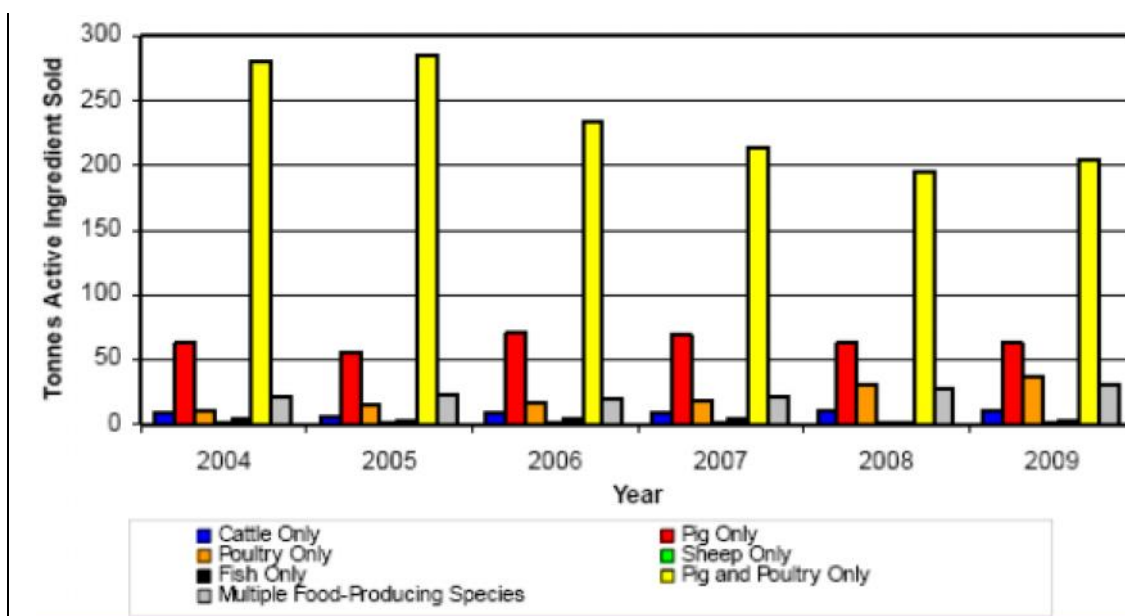
When used correctly animal medicines don't pose a significant risk to human health. There are a number of regulations and laws in place to ensure the correct use of an appropriate efficacious product. All antibiotics for use in animals are classified as POM-V, i.e. they must be prescribed by a vet for animals under their care following a clinical assessment. This helps to ensure that the appropriate medicine is given for the correct infection and is used properly. All medicinal products brought to the market have to undergo a large number of trials to prove their safety and efficacy. Antibiotics available for use in food producing animals have a maximum residue level (MRL) and a withdrawal period which has been set by the appropriate licensing body, in the UK the VMD. The MRL is based on the No Observed Effect Level (NOEL), i.e. the amount of residue in edible tissues and products considered not to present a safety concern for human health, and incorporates a large safety margin. The withdrawal period is the length of time after the end of treatment which a veterinary medicine must pass so that any residues in edible tissues and products are not above the MRL.

In the Dairy industry antibiotics are used to treat disease and to ensure animal welfare. Increasing dairy herd sizes means more cows are being kept in closer proximity creating a greater infectious pressure. Cattle are being bred for production and are being pushed to their metabolic and immunological limits. Modern cows are being asked to produce vast quantities of milk and can suffer from periods of low or negative energy balance and poor immunity making them more susceptible to infection. This alongside a larger infectious challenge means that our modern dairy cows are more at risk of succumbing to disease. Restricted antimicrobial choice will lead to decreased cow health and welfare.

Antimicrobial Usage in the UK

The VMD produce an annual report in which they collect, collate and publish figures on UK sales of antimicrobials (2, 3). From this report we can see that the overall sales of animal therapeutic antimicrobials has decreased by 11% in the last number of years from 454 tonnes of active ingredient to 402 tonnes. There was a corresponding decrease in antimicrobial use in food producing animals from 393 tonnes in 2004 to 349 tonnes in 2009. The figure for food producing animals does not allow for those animals that were treated that did not make it into the food chain. Of the total antimicrobials used in animals 90% of them are used in pigs and poultry. Cattle-only products account for about 3% with multi-species products making up between 8 and 9%. It can be concluded that approximately 6-8% of antimicrobials are used in dairy cattle, 24-33 tonnes.

Figure 1 Total Sales of Therapeutic Antimicrobials in Food Producing Animals 2004 – 2009



Approximately 13 billion litres of milk is produced annually in the UK (2). Not all of this is sold for human consumption; an estimated 183 million litres are fed to calves and other animals or are treated on farm as waste. The quantity of milk produced for each tonne of intramammary product sold has fluctuated – around 0.1 mg of antimicrobial was sold per litre of milk produced. The amount of lactating cow intramammaries fell between 2004 – 2009 as did the amount of dry cow products. There has also been a decrease in cows numbers (7%) over this period and when this is taken into account the amount of dry cow products may have increased slightly but sales of lactating cow products has still decreased, at 1,298 kg in 2009 (2).

Antimicrobial Usage in Europe

The European Medicines Agency's (EMA) mission is to "foster scientific excellence in the evaluation and supervision of medicines for the benefit of public and animal health" (3). As the use of antimicrobial agents is a possible risk factor for the development and spread of antimicrobial resistance, data on the usage of antimicrobials in food-producing animals is needed to identify and quantify developing and spreading antibiotic resistance in animals. Recognising this need the agency set up the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project in September 2009. The project was charged with the task of harmonising the approach for the collection and reporting of data. They produce an annual summary report on the use of antimicrobials across the member states.

A difference in prescribing patterns was seen across the 9 countries. This could be due to the availability of vet products, prices, risk-management measures implemented, vets, animal production systems and the general situation in the various countries with regard to infectious disease. France sold the most tonnes of active ingredient in 2009, 1,064 tonnes. However, when the figures were corrected per Population Correction Unit (PCU) Netherlands came out on top with the most active ingredient sold per PCU. (See table 1)(3)

Table 1 Sales normalised by population correction unit (PCU) for the years 2005-2009. Data are expressed as mg/PCU

Country	2005	2006	2007	2008	2009
Czech Republic	103	113	101	114	106
Denmark	46	45	47	46	53
Finland	24	25	27	32	32
France	169	164	173	154	141
Netherlands	160	169	179	168	165
Norway	14	15	15	14	14
Sweden	20	21	21	20	19
Switzerland		93	99	99	95
United Kingdom	72	65	64	63	68

The pattern in Europe is a similar one to the UK where we see an overall decrease (11.2%) in the tonnes of antimicrobials sold between 2004 and

2009. As we saw from looking at sales of dry cow antibiotics it is important to take the animal population into account to be able to look at the true picture. When the data was corrected per PCU the decrease seen was 8.2%. This decrease was mainly attributable to the decrease in sales of tetracyclines (3, 4).

CONCERNS

There have been a number of measures implemented already in some European countries as we will discuss later but as of yet no definitive restrictions have been implemented in the UK. The issue of antimicrobial resistance is an emotive one and it is vital that decisions are not just based on emotion and fear but on science. The majority of antibiotics used in animals are in the pig and poultry sector where in-feed and in-water medication is wide-spread (3). The route of administration contributes to the risk of developing resistance – the oral route being much higher risk than injectable. The majority of antibiotics used in cattle are injectable, in the case of the dairy industry the intramammary route is also widely used.

The UK

There are a number of bodies in the UK that monitor and advise on the responsible use of antibiotics. The BVA (5) have created the 8 Point plan where they give guidelines on the responsible use of antibiotics. The use of bacteriology and sensitivity are part of their guidelines and they also recommend reporting any suspected lack of efficacy to the VMD.

The 8-point plan:

- Work with clients to avoid need for antimicrobials;
- Avoid inappropriate use;
- Choose the right drug for the right bug;
- Monitor antimicrobial sensitivity;
- Minimise prophylactic use;
- Minimise use perioperatively;
- Record and justify deviations from protocols;
- Report suspected failure to the VMD.

It has been widely debated whether or not any form of antimicrobial advertising to farmers should be allowed – as of yet the promotion of antimicrobials to farmers is permitted but with stricter guidelines as set by NOAH in July this year (6). The emphasis in any promotional material to farmers should be educational with a disease based focus, more product detail is required including withdrawal periods, the strap line “use medicines responsibly” must appear and contact details for further information must also be shown.

The Responsible Use of Medicines Alliance (RUMA) is an unique initiative involving organisations representing every stage of the "farm to fork"

process. RUMA aims to promote a co-ordinated and integrated approach to best practice in the use of medicines (7). It is a non-profit making organisation set up in November 2007 to promote the highest standards in food safety, animal health and welfare. RUMA publish guidelines on the responsible use of medicines for all the major food producing species, these guidelines are continuously updated according to ongoing developments in the industry.

Dairy UK is an organisation that represents the UK's dairy industry. Following on from an MSD Animal Health facilitated meeting of various industry members there has been a task force set up at Dairy UK to look into the issues of bulk milk tank failures and why they occur, and also to look at the science behind antimicrobials.

Europe & Elsewhere

In the last couple of months several reports were published on the use of antimicrobials in animal in Europe in general and dairy more specifically, e.g. the EMA report previously referred to. There have also been reports from the Netherlands, e.g. the LEI report, and from Denmark, e.g. the DANMAP report.

In the Netherlands a target of 50% reduction of antibiotics by 2013 has been implemented. No new guidelines have been given on how to achieve this reduction, but existing initiatives will be supported - the most relevant ones being the formularies (antibiotic therapy selection and use guidelines by Royal Dutch Society of Veterinary Medicine) and Vetbase, an electronic system tracking all antibiotic prescriptions of the vet and use by the farmer, which allows for benchmarking at both the vet and farmer level. Objectives for reduction (based on a calculation of Defined Daily Dosages) have been put in place and thresholds for a correction plan for heavy-users has been set.

In Denmark and France there has been a voluntary 2 year moratorium on the use of third and fourth generation cephalosporins in pigs. In the past month in Germany there has been massive media attention focussed on the use of antimicrobials in animals and the issue of antibiotic resistance and the consequent public health concerns.

The debate on the use of antibiotics in animal production is coming more and more to the forefront in the media in the US. Legislative initiatives to ban non-therapeutic uses of antimicrobials critical for human therapy have been re-initiated by Louise Slaughter (8). Several major producers have already taken pro-active steps regarding the use of antibiotics. A process of public hearings has started on reporting on antibiotic use in livestock.

Elsewhere (Australia, India, Thailand) the debate on the use of antibiotics, classified by WHO as critically important for human therapy, and more in general the use of all antibiotics in animal production is increasing.

MSD FACILITATED GROUPS

UK Residue Workshop

Early in 2011 MSD Animal Health took a group of stakeholders to their research facilities in Schwabenheim to discuss the issues surrounding bulk milk failures as a result of suspected residues and to understand further the science behind the development of antimicrobials. This group included farmers, vets, processors and dairy consultants, all of whom can be affected by these issues. A tour of the facilities took the group from the concept stages of new antimicrobials, including a 3D computer modelling which rivalled the best 3D movies, through the first production of a single molecule of antimicrobial, all the way to a final full production process. The group received a presentation from the VMD which explained the science behind minimum residue levels and withdrawal times, from vets about their decision processes when prescribing antimicrobials, and from a farmer explaining how antimicrobials had an essential role on the farm. The group then discussed the difficulties faced by farmers when they experience milk failures. Many of these are down to human error or accidents, but some remain unexplained. The tests to detect antibiotics in milk can be overly sensitive to certain antimicrobials while not being sensitive enough for others, while their specificity can sometimes be a problem with some failures occurring when there are no residues. It was felt that further education and guidelines for farmers would be useful. This initial meeting has now led to a task force being started at Dairy UK to look further at these matters.

UK National Mastitis Panel

Annually MSD Animal Health organise a meeting for a group of enthusiastic udder health experts. Each year a member of the group chairs the meeting and volunteers from within the group present on work they are doing or have done in the past or present on a current relevant topic. There are normally 2 or 3 presenters, with short presentations to allow the main emphasis to be on the following discussions.

This year Dr Andrew Bradley, MA VetMB DCHP DipECBHM PhD MRCVS, reviewed a trial (9) he had done previously on the use of a teat sealant with and without a dry cow antibiotic. The main discussion arising from Dr Bradley's presentation was the pros and cons of blanket use of antibiotics at dry off versus selective use. It was acknowledged within the group that the dry period and dry cow therapy is very important for the control of contagious pathogens. However, the reduction in use of dry cow antibiotics in low SCC herds where environmental pathogens are the predominant pathogens by using selective treatment rather than blanket dry cow antibiotic therapy was considered. This led to a lively discussion on whether

or not this would result in more contagious pathogens being implicated in cases of mastitis and a corresponding rise in SCC in these herds.

As with many areas of cattle health, nothing is ever straight forward or simple, Dr Bradley pointed out. With or without DCT, during the last part of the dry period the same numbers of infections are picked up but the pathogens/aetiology is different. There is little doubt, he stated, that the use of antibiotics during the dry period decreases SCC, decreases Staphs but possibly results in an increase in the rates of coliform mastitis.

What was agreed within the group was that there was a need for guidelines on dry cow treatment and also on antibiotic use in general. There was much discussion on how to communicate the importance of having science involved in any guidelines on the use of antibiotics and indeed how to communicate any guidelines themselves to the wider audience. This led on nicely to our second speaker.

Arising from feedback and ideas from the group last year, communication was highlighted as a key area of interest and of importance to the industry. As a result, this year for the first time a speaker from outside the group, Jolanda Jansen, a communications expert, presented and led a discussion on how to encourage or compel people to comply with given instructions. Referring to a quote from Confucius, she challenged the group to adopt a mantra: *Tell me and I will forget, show me and I might remember, involve me and I will understand*. Working in this way with people will bring all the different types/mindsets/characters along with you and, she told the group, that there will no longer be the 'hard to reach' person. Anticipating mindset is essential as it will inform the way in which you speak to people, question them and advise them. Once an understanding of the type of person you are dealing with is established, the next phase of influencing them can be the target.

THE FUTURE

Communication and education around the responsible use of medicines, and the science behind antimicrobials and resistance, is essential. The future of antibiotic use in animals is unclear. There are many possible scenarios: continue as we are, stricter measures with regard to antibiotic use and record keeping, a compulsory reduction similar to the one that has been put in place in the Netherlands, a compulsory reduction or ban of certain antibiotic classes, restricting the range of antibiotics available to use or a general ban on all antibiotics for use in animals. Knowledge is key to making the correct decisions. The industry needs to look at the whole picture, but while the science is essential, the emotions and fears of the general public must be taken into account. We need to work together in a concerted effort to ensure antibiotics are used correctly and responsibly and that the health and welfare of our animals is taken into account.

CONCLUSION

In recent times there has been a lot of discussion by scientists, politicians and the media on the use of antimicrobials in humans and in animals and more specifically on antimicrobial resistance. The spread of antimicrobial resistance has become a global concern with resistance known to occur in both humans and animals. However resistance is a natural phenomenon and the factors that drive its development are unclear. As of yet the evidence would suggest that the use of antibiotics in animals has had little or no impact on the incidence of antibiotic resistance in human infections. However there is a serious political risk to the future use of all antibiotics in veterinary medicine and the industry needs to be involved in any discussions around the use of antibiotics in animals and any possible restrictions that may be implemented. Ultimately, antibiotics are vital to the health and welfare of our animals as well as that of humans.

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- 5 BVA website – www.bva.co.uk
- 6 NOAH website - www.noah.co.uk
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NOTES

MASTITIS IN LARGE HERDS – A VETS VIEW

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INTRODUCTION

The UK average herd size in 2009 was 113 cows (Dairy Statistics, DairyCo); it seems inevitable that herd size will keep increasing and many veterinary practices in the UK attend herds of 500 plus cows. So what is a large herd - above the national average, over 200, 500 or 1000 cows? In this paper I will consider the situation in an 800 cow herd but also part of a group of herds under one management totalling 2500 cows.

Regardless of the definition, as herd size increases there will be a decrease in labour units/cow and often there may be increasing layers of management and the use of 'advisors', and herein lies the challenge.

THE VET'S ROLE IN MASTITIS CONTROL

Historically (and too often currently!) we as vets are employed in a reactive way in mastitis control i.e. treating severe cases, selling tubes and occasionally trouble shooting cell count problems. Modern pro-active veterinary services have changed this considerably and now help the farmer to sell high quality (low cell count, low bactoscan) milk with as little discarded due to mastitis treatment and as few cows culled for mastitis as possible.... and all as economically as possible!

The modern vet practice now has more information about their clients' farms than ever before. With respect to mastitis control regular milk company statements, assuming herds milk record data from NMR Companion or CIS websites, data can be analysed through software programs like Interherd or Total Vet and there is the back up from internal practice laboratories or external labs such as AHVLA, QMMS, NML, Gloucester Laboratories etc.

Excellent CPD helps us build on our knowledge base and schemes such as the DairyCo Mastitis Plan provide excellent frameworks for mastitis investigation and monitoring. Good liaisons with the milk machine professionals e.g. The Dairy Group and other advisors such as the nutritionist complete the 'team'.

As long as the farmer is enthusiastic there should be no excuse for not making progress!

CHALLENGES OF THE LARGE HERD

In my experience, whilst the principles of mastitis control are the same be it a large or small herd, the large herd presents some particular challenges.

Unless employed by the farm a senior vet is likely to have other responsibilities with regard to helping run his own business, develop more services, attend to other clients etc. Time is often in short supply but time must be made to understand how the farm operates, what is changing as well as keeping on top of the mastitis data (the measure, monitor and respond part).

Time 'sold' to the client must be value for money. Therefore all procedures, monitoring systems, meetings etc. must have useful outcomes. Lay staff can be used to process data and produce reports providing they are trained. Meetings should be planned with an agenda and chaired well – this may require flexibility on the vet's part to make him/herself available. Taking staff away from the farm environment can mean a more focussed discussion without the distractions that inevitably crop up all the time on site. Keeping regular contact is important – e-mail and text messaging are really useful but also vital is to talk directly to the appropriate people. No vet is indispensable, no vet knows it all and no vet suits every farmer – this is not the role for a vet with a large ego! I have a 'buddy system' on all the farms I visit – my number 2 know what is going on, is copied in to all communications, attends meetings when possible, covers when I am unavailable and will eventually be number 1!

A large herd/dairy business will have many people involved in the operation; owner(s), herd manager (trainee manager), herdsman, milkers, consultants, technicians etc. Communication therefore becomes a challenge but must be achieved.

As well as being 'the vet' my roles seems often to include being 'agony aunt', conflict resolution and 'educating, persuading and cajoling' as well as co-ordinating meetings!

Once agreements are reached and procedures and protocols are established ensuring compliance is important and new staff arriving at the farm with preconceptions and fixed ideas can be a particular challenge and require careful handling to get them 'on board'.

Despite the close involvement with a dairy business unless employed directly it is worth remembering that the vet is the health advisor not the health manager. Attempts to manage the health are likely to fail without daily presence on the farm but more importantly the vet is likely to be performing the role (badly!) for which someone else is being employed and should be encouraged to do – not a good economic solution for the farmer!

MASTITIS CONTROL AT JF COBB & SONS

Data handling and analysis is crucial. Clinical records are transferred on a monthly basis to the practice via an Interherd file and cell count data is taken from Herd Companion. From this a bespoke monthly report is produced including mastitis data (but also fresh cow health, fertility, lameness, nutrition). A specific cell count action list is also produced. On a quarterly basis data is analysed through Total Vet and any shift in mastitis patterns noted.

Cow side the parlour is checked regularly by a consultant from The Dairy Group, both a static and dynamic test that includes observation of the milking routine. Six monthly teat scoring is carried out to support these investigations by the vets.

We believe heavily in investing time in the staff and regular 'coaching' sessions are carried out with the milking team (4 times/year, they have to attend at least 3). This may be office based knowledge transfer e.g. how the milking routine can influence mastitis or may be in the parlour or cubicles discussing particular tasks. Positive reinforcement and encouragement is key to these sessions with simple graphs showing improvements in mastitis a useful visual tool.

All procedures are described in clear protocols (translated as appropriate). New staff must be trained to follow the various protocols – we believe that if someone is asked to do something they should understand why they are being asked to do it.

MASTITIS RISK FACTORS, INTERVENTION AND OUTCOMES

Attending to mastitis risk factors involves a whole farm approach and the DairyCo mastitis plan is a useful framework. Essentially though we need our cows to be clean, comfortable and well fed and the ABC (air, bunk, comfort) of assessing a unit is a good place to start, with a few mastitis specific areas e.g. in the parlour, added on.

Different units will have different limitations –target levels for one will therefore have to be different from another or the target can become demoralising. Targets are set as 'number of cases/month'; this is easier for staff to understand than rolling incidence figures whether it be cases/100 cows or cases / 100 cow-years!

Interventions based on risk assessment should be prioritised and the 'team' encouraged to take ownership of the solution, which is arrived at through knowledge transfer and discussion. Meetings and discussions should whenever possible have a conclusion with positive action. Outcomes should be based on the best evidence available – this is the only secure way to

establish procedures and protocols. Decisions based on anecdotal evidence or worse can potentially have disastrous economic consequences.

As an example we attempt to have a single treatment protocol in use across all the dairies – there is little evidence to support any particular treatment so why have 5 different ones? All managers must understand why and agree to the protocol; once in place success is monitored and things can be changed as required. All protocols have a review date and it is crucial that they are seen as ‘current’ working documents not some ageing laminated sheet that no one can remember when it was put up!

THE FUTURE

Two current issues we are addressing are antibiotic usage and vaccination.

There is increasing interest by the supermarkets in the antibiotic resistance debate. Following discussions the decision was made to no longer use fluoroquinolones and reduce the reliance on 3rd and 4th generation cephalosporins (by increasing the use of selective dry cow therapy and using alternative tubes when needed). Any use of cephalosporins for other conditions is done strictly according to protocols.

Mastitis vaccination; is it value for money? This is under continued debate, as ever on farms many things change at the same time and this is a typical situation where good evidence is needed. Hopefully before too long there will be good data from UK trials.

ACKNOWLEDGEMENTS

I would like to thank the owners and all the staff at JF Cobb and Sons and Winfrith Fields Farm LLP for their enthusiasm and positive input into the health care of their cows. I am privileged to work with such forward thinking people. Thanks also to the staff at Synergy Farm Health, particularly ‘Viv’ without whom I would spend far too many late nights at my desk.

NOTES

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POSTERS

USE OF THE 3M™ CLEAN-TRACE™ SURFACE ATP TEST TO ASSESS PARLOUR HYGIENE AND INVESTIGATE BACTOSCAN BREAKDOWNS

Judith Roberts & Matthew Haslam

Lambert, Leonard and May Farm Vets, Old Woodhouses, Broughall, Shropshire, SY13 4AQ

SUMMARY

The 3M Clean-Trace Surface ATP test is a simple immediate swab based system for measuring cellular ATP (adenosine triphosphate) levels on surfaces. The swab is used on a 5cm² dry area and then added to the enzyme reagent in the container to allow a fluorescence measurement of the ATP levels to be performed on farm using a hand held analyser.

TP is a coenzyme used as an energy carrier in the cells of all known organisms, the swab result is therefore a quantitative indicator of metabolically active cells on any surface. The cells of interest in this investigation are bacteria, but ATP released by skin cells will also affect the swab result. The 3M Clean-Trace Surface ATP test is extensively used in the food industry to assess the cleanliness of food preparation areas, and also in hospital wards and theatres to assess hygiene levels.

We have used this innovative technology to assess hygiene on dairy farms in two separate situations; one involving bulk milk Bactoscan problems, the other to evaluate the efficacy of pre-milking teat disinfection as part of a parlour hygiene assessment. This novel parlour tool has improved parlour hygiene and is an excellent aid to engage farmers in tackling cleanliness issues on farm.

Bactoscan Breakdowns

In these scenarios the instant result seen with the 3M Clean-Trace Surface ATP test has proved invaluable. Each section of the milking plant is swabbed after the cleaning routine and the level of ATP (and therefore bacterial load) instantly quantified. The level of cleanliness that can be achieved is dependent on the surface being tested, with rubberware harbouring more bacteria than stainless steel, particularly when aged. The ATP value is instantaneous and quantified, the farmer does not need to wait for culture results to see where the problem lies or which areas are not cleaning sufficiently. Examples of the values found are shown in the table below. Although these results will only highlight Bactoscan problems arising from the areas that are sampled, it is beneficial to rule out areas of contamination based on negative swab results. The section of the milking plant that has demonstrated a high ATP result can be thoroughly cleaned (e.g. plate coolers) or altered accordingly (blind ending pipes, corroded rubberware) to reduce the bulk milk Bactoscan.

Pre-Milking Teat Disinfection

The importance of thorough pre-milking teat disinfection in mastitis control has long been established yet the implementation of good teat preparation on farm can often be met with opposition, mainly due to the potential impact on milking times. The 3M Clean-Trace Surface ATP machine has proven to be excellent in demonstrating the effectiveness and importance of pre-milking teat preparation. The use of the swabs on skin will always be higher than inert surfaces due to the high levels of ATP harvested from perfectly clean skin. To establish a target threshold level for clean teat skin the ATP level of teats across a range of farms were measured. This varies depending on the teat preparation routine employed, particularly if teats are left wet allowing water droplets to spread bacteria that are detected by the swab. The threshold quoted below is a rough guide based on the swabs results obtained through this trial. Farms with higher swab levels than the threshold have poorer teat cleanliness (or wet teats) and intervention is considered beneficial. The swab sample can be repeated following additional steps in the pre-milking routine to demonstrate rapidly the results that are achievable on the farm in question. This novel approach encourages farmers to be proactive and involved in improving parlour hygiene with real time quantitative data to assess the improvements as they are made.

Using the thresholds in the Table 1 it is simple, fast and practical to evaluate the hygiene of the parlour equipment and assess the efficacy of the current teat preparation.

Table 1 Surface ATP swab results

Swab site	Suggested threshold	Normal farm results	Problem farm results
Liner	<1000	280-896	2,700-30,000
Teat after preparation	<5000	510-4,600	5,700-55,000
Liner of dump bucket	<1000	300-880	3,100-50,000
Dump bucket	<500	45-320	1,100-72,000
Parlour rubberware	<500	20-250	620-11,900
Vacuum line	<500	11-115	720-1,150
Bulk tank	<50	3-26	115-4900

The use of the 3M Clean-Trace Surface ATP test to demonstrate the hygiene and cleanliness aspect of mastitis control in the parlour has proved to be highly motivating and thought-provoking. It has resulted in significant changes on farm, and has reduced mastitis incidence rates and rapidly corrected Bactoscan breakdowns on the trial farms.

The authors would like to thank 3M for loan of the equipments and swabs and their support during this trial.

BACTERIAL MIGRATION THROUGH THE TEAT CANAL RELATED TO LINER ACTION.

Derek Forbes¹ and W. Gehm².

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Udder health and behaviour of cows being milked are directly related to the performance of milking machines. Observations of milking animals reveals common problems of teat swelling, kicking/stepping, increased heart rates, increased adrenaline, uneven udders and slow milking quarters associated with the milking process.

Mastitis is the most costly problem in the dairy industry with cleanliness and procedures identified as a means of achieving quality milk and positive animal health but the mechanisms by which bacteria cross the teat canal are not fully understood. Research (DF) showed that skin bacteria, which include *S. aureus*, can remain confined in the teat canal for prolonged periods. Teat sinus milk collected by syringe through the teat wall could be sterile but when collected conventionally was then contaminated by the bacteria in the teat canal. It was postulated that the pinching action at the teat apex by the liner could cause an intrusion of the wax-like keratinaceous lining transporting bacteria into the teat sinus, thereby introducing an intra-mammary infection and possibly mastitis.

A unique milking machine (CoPulsation™,(WG)) was developed further supporting this idea. It has been determined that a combination of a very short pulsation C phase with a low collapse force round liner changes the closing liner action. This liner action simulates a full teat length massage similar to the suckling action of a calf as opposed to the common pinching action. The combination of liner action and vacuum cause milk flow from the teat/udder and create the environment the cow experiences while milking. The pulsator is at the core of a basic milking system with the dynamics of air and vacuum from the pulsator interacting with the liner playing a key role in the final results.

Experience with the use of a short C phase pulsation system shows that the milking environment for the animal is improved. Data shows it lowers both the incidence of mastitis and SCC levels, substantially reduces the stress factors to the cow eliminating teat swelling, reduces the time of and improves complete milk-out while retaining the natural integrity of the teat canal.

STRATEGIES TO REDUCE MILKING DURATION OF INDIVIDUAL COWS

Paul Edwards and Jenny G. Jago

DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand

An individual cow's milking duration is an important factor in determining how long it takes to milk a herd. In pasture based systems milking can account for more than 50% of labour on farm (1). As herd size continues to expand herd milking duration will increase, therefore impacting the labour requirement. Strategies to reduce individual cow milking duration are thus important to manage herd milking duration and labour requirements.

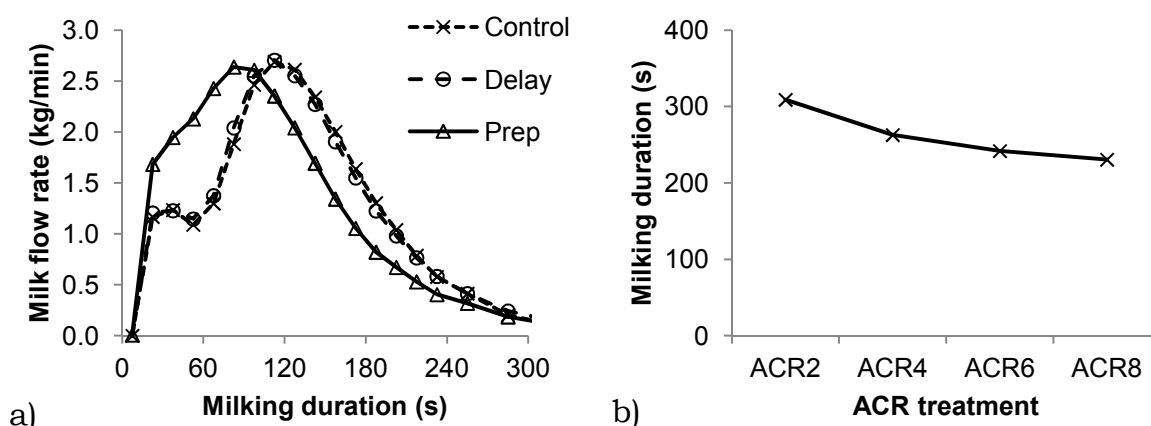
A study was conducted in late lactation on DairyNZ's Lye Research Farm (Hamilton, New Zealand) using 96 mixed age Friesian-Jersey cross cows not accustomed to a pre-milking routine. Cows were milked in a 30 bail rotary dairy. Three pre-milking routines were selected to assess the impact of pre-milking stimulation on milking duration. The first routine was a control (Control), where the clusters were attached at the first bail with no other preparation. The second treatment was tactile stimulation (Prep) by removing two squirts of foremilk from each quarter followed by cluster attachment 60 s after entering the bail. The time taken to remove foremilk was around 15 s, resulting in a 45 s delay before cluster attachment, which was appropriate for cows with a low degree of udder fill (2). The third pre-milking treatment was delayed cluster attachment only (Delay), where clusters were attached 60 s after entering the bail. With each pre-milking strategy four automatic cup remover (ACR) thresholds were imposed: 0.2 kg/min (ACR2), 0.4 kg/min (ACR4), 0.6 kg/min (ACR6) and 0.8 kg/min (ACR8). Cows experienced each pre-milking routine for 2 weeks, but remained on their allocated ACR treatment for the 6-week trial.

Individual cow milk yield, milking duration (cluster on to cluster off time), average milk flow rate, maximum milk flow rate, time to mean milk flow rate, time from maximum milk flow rate to end of milking and the milk flow rate at 15-60 s intervals for 10 min was recorded at each milking session. Milk samples were collected weekly to determine composition and somatic cell count (SCC). Post-milking strip yield was measured on 3 occasions.

Data were analysed using mixed models. SCC data were normalised using a \log_{10} transformation, and strip yield data normalised using a square root transformation.

Cows receiving Prep treatment had a shorter milking duration than the Control (18 s) and Delay treatment ($p < 0.001$), which both had bimodal shaped flow curves (Figure 1a) that did not differ from each other. Time to mean milk flow rate was lower ($p < 0.001$) and the time from maximum flow rate to end of milking was greater ($p < 0.01$) for the Prep routine. Milk production, maximum flow rate, strip yield and SCC were not affected by treatment ($p > 0.42$).

Figure 1 Milk flow profile of three pre-milking routines (a) and the effect of ACR threshold on average milking duration (b)



In comparison to the ACR2 treatment, the milking time per cow on the ACR8 treatment was 79 seconds less ($p < 0.001$; Figure 1b). Milk production variables and SCC were not affected by treatment ($p > 0.25$). An increase in strip yield of 0.3 kg was recorded with increasing ACR threshold ($p < 0.05$).

The results achieved from stimulation and increased ACR thresholds can be used to devise effective strategies to reduce herd milking duration. The time cost of applying the Prep routine (60 s) was more than the reduction in cow milking duration (18 s). The Delay routine results contradict guidelines to stand several bails from the entranceway (3). Thus, neither form of pre-milking routine gives an advantage in reducing overall milking time with cows bred for minimal milking routines.

To take advantage of the reduction in cow milking duration from higher ACR thresholds and to shorten herd milking duration, platform speed must be increased in rotary dairies or work routines sped up in herringbone dairies. Alternatively, for farmers building new dairies, a smaller size (fewer bails) may be constructed to achieve the same throughput for less capital cost.

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ACKNOWLEDGEMENTS

This study was part of a programme of research funded by DairyNZ on behalf of all New Zealand dairy farmers.

MONITOR THE LEVEL OF CHRONIC HIGH CELL COUNT COWS TO AVOID PENALTIES

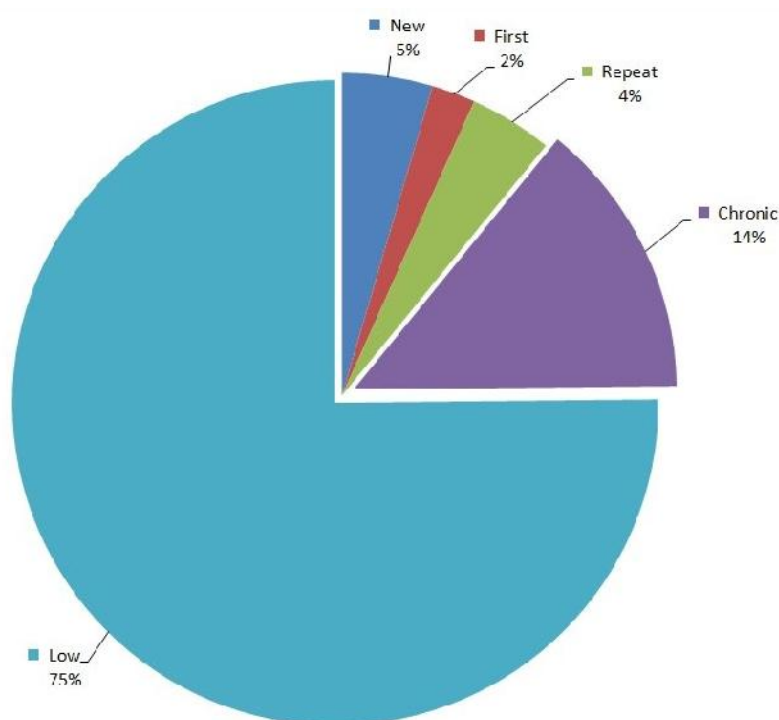
James D Hanks¹ and Chris Watson²

¹Veterinary Epidemiology & Economics Research Unit (VEERU), School of Agriculture, Policy Development, The University of Reading, PO Box 237, Reading RG6 6AR, UK. E-mail: james.hanks@panveeru.net; ²Wood Veterinary Group, 125 Bristol Road, Quedgeley, Gloucester GL2 4NB, UK.

An analysis of somatic cell counts in **6.7 million** milk samples analysed by National Milk Records (NMR) in 2010 showed that 75% of milk samples were below 200,000 cells/ml. This was the third consecutive year of improvement in the percentage of low cell count milk samples.

Each milk sample was assigned a Herd Companion category to denote the duration of high SCC levels of the cow. The category “Chronic” indicates a milk sample from a persistent high cell count cow that also had a high cell count (>200,000 cells/ml) at the previous milk recording. The distribution between Herd Companion categories is given in Figure 1.

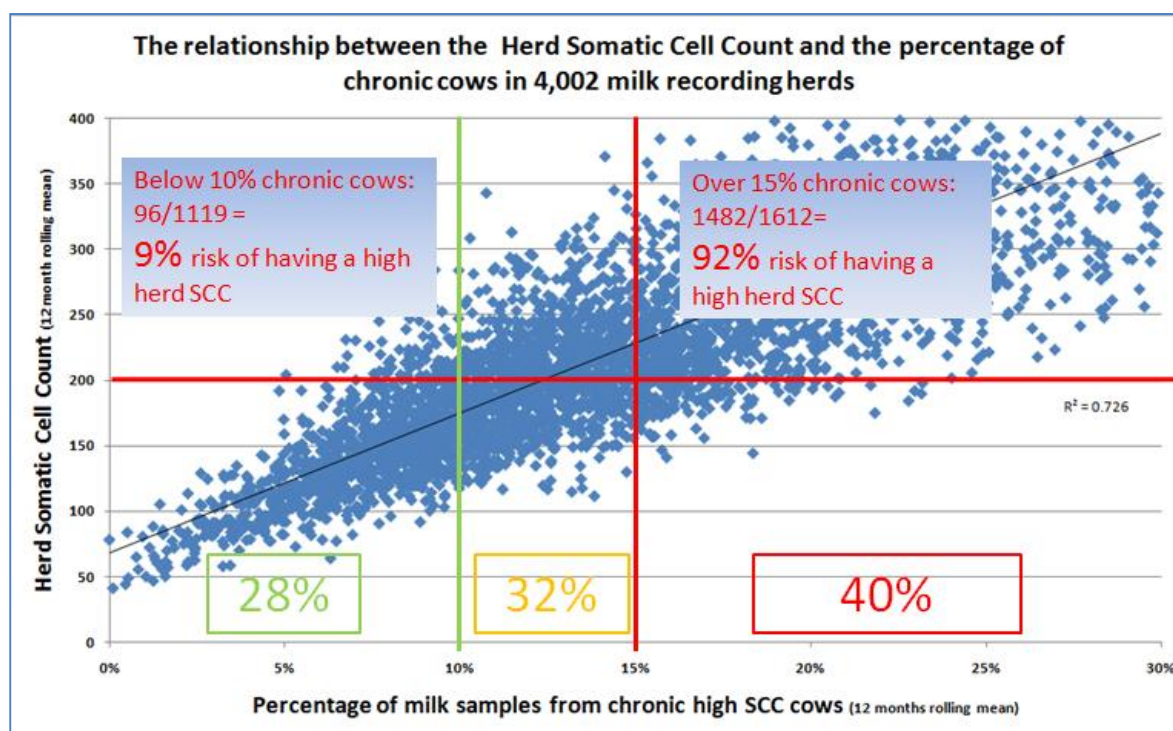
Figure 1 Distribution of 6.7 milk samples between Herd Companion categories



14% of all milk samples in 2011 originated from persistent high cell count cows. Of all high cell count samples **56%** were from the **chronic** category cows.

An analysis of 4,002 herds compared the rolling 12 month **herd somatic cell** count against the percentage of milk samples originating from **chronic** category cows. The strong correlation ($R^2=0.73$) is shown in Figure 2.

Figure 2 Correlation between the % of chronic cows in a herd and the overall herd cell count



In four of every ten herds over 15% of milk samples are derived from chronic, persistent high cell count cows. At this level of chronic cows 92% had a herd cell count above 200,000 cells/ml, a common penalty level. In contrast, herds with below 10% chronic cows had less than 10% of herds with a herd SCC in excess of 200,000 cells.

Farmers need to be better informed of the damage caused by maintaining persistent high cell count cows. As well as ensuring effective cure and protection in the dry period this requires greater emphasis on preventing emerging cell count infections (New, First at Repeat categories) extending in to chronic infections.

MASTITIS BACTERIOLOGY AND qPCR - MYTHS AND REALITIES

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INTRODUCTION

The diagnosis of the aetiology of infectious bovine mastitis is a challenging area and has historically been achieved using conventional bacteriological techniques. More recently the advent of molecular techniques has offered the opportunity to explore and develop both novel and supplementary diagnostic techniques such as PCR, qPCR, MALDI-TOS MS and micro-arrays. The first such diagnostic technique to become commercially available is qPCR as offered through the PathoProof™ Mastitis PCR Assay offered by Finnzymes. This assay is now offered as an alternative to bacteriology by a number of commercial laboratories. The purpose of this paper is to address some of the questions frequently raised to the authors by practitioners, advisors and farmers.

WHAT IS PCR AND HOW DOES THE TEST WORK?

Polymerase chain reaction (PCR) is the process by which a DNA sequence can be amplified to the point of detection. The reaction relies on relatively small pieces of artificially created DNA (primers) made up of 'unique' sequences, sticking (annealing) to their equivalent region on the bacterial genome. An enzyme then makes a copy of the gene sequence and the process is repeated. In this case the gene of interest is that coding 16S RNA which is important in protein production in bacterial cells. Whilst overall this gene is highly conserved the exact make up varies between different bacteria and therefore the ability to differentiate different species exists.

IS PCR MORE SENSITIVE THAN BACTERIOLOGY?

This question is almost impossible to answer. Many of the comparisons done between PCR and bacteriology have not used the most sensitive bacteriological techniques (2,4) making it difficult to draw conclusions. If the bacteria in the milk sample are dead then PCR is obviously more sensitive as the bacteria will not grow when cultured. However, when detecting viable bacteria, arguably bacteriology may well be more sensitive. The 16S RNA gene is an obvious choice for a PCR assay as there are usually multiple copies of this gene in each bacterium (typically 5-7: <http://rrndb.mmg.msu.edu/index.php>); however 1,000 - 10,000 copies of this gene are necessary for the PCR to detect the pathogen of interest (1). Given that 2 mL of milk are used in the assay there needs to be a minimum of between 75 and 1,000 bacteria/mL (viable or dead); in contrast well conducted bacteriology can detect as few as 10 viable bacteria/mL of milk.

SURELY PCR DETECTS ALL THE MAIN AND THEREFORE IMPORTANT MASTITIS PATHOGENS?

The main Pathoproof™ assay can detect 12 species of the major mastitis pathogens as well as the minor pathogens *Corynebacterium bovis* and the Coagulase -ve *Staphylococci*. Whilst these pathogens encompass many mastitis cases the list is far from exhaustive. In a recent study 101 different species were identified amongst 1307 isolates (data on file) with more than 45 different genera being routinely identified in our labs. Whilst arguably many of the key pathogens are covered the key is in interpretation of results.

HOW DO I INTERPRET THE RESULTS OF THE PCR TEST?

This is the single biggest challenge facing the use of this test and is an area in which we have much left to learn. Perhaps the single biggest drawback is the lack of any marker for contamination. In the absence of a 'broad scan' as provided by culture it is impossible to know if the pathogens identified (especially if they are environmental in nature) by PCR are causal or merely contaminants. Scrupulous sampling technique is an absolute pre-requisite and even then this problem still exists. There are also some doubts around the use of the 16S RNA gene sequence for speciating some bacteria, particularly the *Staphylococcus* spp (3) meaning that more work is needed before we can be confident that identifications in this area are robust.

HOW USEFUL IS A BULK SAMPLE SCREEN?

There may be merit in using the PCR test to screen herds for the presence of *Streptococcus agalactiae* and *Mycoplasma* spp, though these pathogens as causes of mastitis are relatively rare in the UK. The use of the standard assays (bacterial culture or PCR) for screening bulk milk is **not** informative and is at worst misleading. Quantitative differential counts by bacterial culture however can help identify sources of bacteria found in bulk milk

CONCLUSION

Molecular techniques undoubtedly offer real opportunities to advance mastitis diagnostics, but currently should be viewed as supplementary to rather than as a replacement for conventional bacteriological techniques.

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ASSOCIATION BETWEEN HERD SIZE AND SOMATIC CELL COUNT FOR IRISH AND UK DAIRY HERDS

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INTRODUCTION

European Union milk quotas are to be phased out by 2015, and the importance of producing low somatic cell count (SCC) milk for dairy farmers trading on the world market is expected to increase to counter volatile prices (1). Milking more cows per herd is a well established strategy to increase production efficiency (2), and this trend is set to continue. The aim of this research was to investigate the impact of herd size on cow level test day SCC with emphasis on comparison between cows in Irish and UK herds.

MATERIALS AND METHODS

The study populations were 7,608 Irish dairy herds, with 10,316,907 records from 869,593 cows taken between 2005 and 2009, and 2,128 UK dairy herds, with 6,793,298 records from 474,678 cows taken between 2004 and 2006. Two samples of 500 Irish, and 200 UK herds were taken at random. Four-level linear models for test day log SCC were developed using data for herds in the first samples; random effects structure accounted for clustering of cows within herds, parities within cow, and recordings within parity. Data from the second sample datasets were used for cross validation of the models.

PRELIMINARY RESULTS AND DISCUSSION

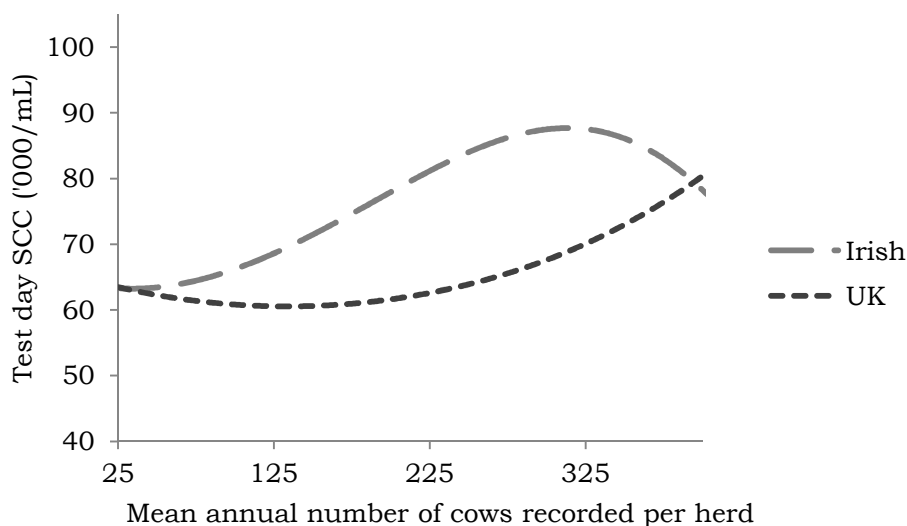
Factors influencing test day SCC were herd size (Figure 1), calendar month and year of test, parity, stage of lactation, and test day milk yield adjusted for fat and protein content. Model fit was acceptable.

Up to annual herd sizes of around 180 cows, test day SCC for cows in Irish herds increased, but remained constant for cows in UK herds. For herd sizes over 180 cows, test day SCC increased in UK as well as Irish herds, and the rate of increase in the 2 countries was approximately equal from 225 cows. For Irish herds over 316 cows, mean test day SCC decreased although for cows in UK herds there was continued increase.

These results suggest that many Irish and UK dairy herds have failed to benefit from economies of scale for mastitis control. Further investigation is required to determine underlying reasons for the differences between Irish

and UK herds, and to develop strategies to optimize udder health during future dairy herd expansions.

Figure 1 Association between annual herd size and cow level test day somatic cell count (SCC) for Irish and UK herds¹.



ACKNOWLEDGEMENT

Simon Archer is supported by a Teagasc Walsh Fellowship.

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¹Refers to parity 2 cows, 5 days in milk, with mean test day milk yield, fat, and protein, during October 2005.

NOTES

NOTES

APPENDIX

NATIONAL MASTITIS SURVEY

National Mastitis Survey Results

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² Walton Manor, Walton, Milton Keynes, MK7 7AJ

The National Mastitis Survey has taken place in 2009, 2010 and 2011 with over 1000 farmers participating each year.

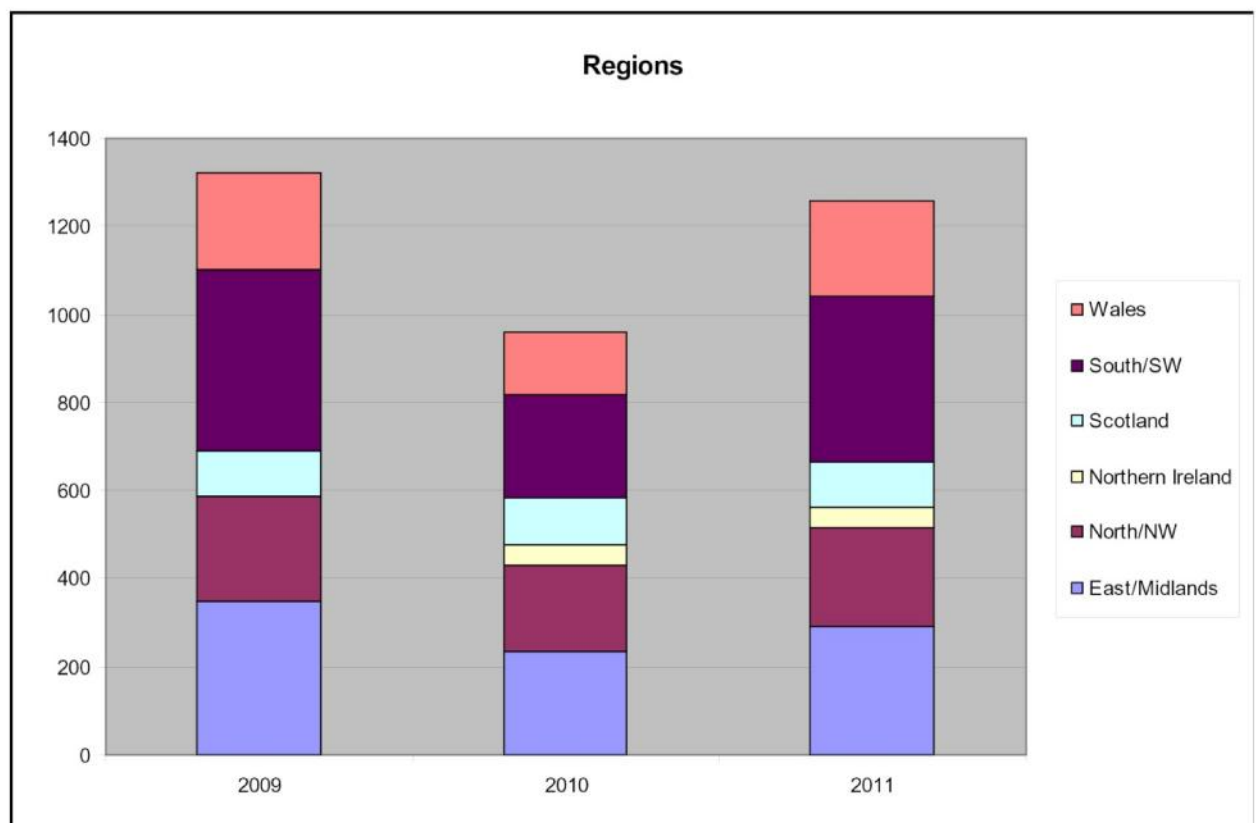
The survey aims to establish a true representation of the on-farm situation and the challenges faced by farmers on a daily basis by asking between 20-25 questions in a multiple choice format.

Key findings

- Mastitis remains a huge challenge on the majority of dairy farms
- Bacteriology rates low
- Herd yields have increased esp in larger herds
- Mastitis case rates seem to be increasing
- A greater proportion of low BMSCC herds foremilk
- Great improvements have been made in pre- and post-milking teat preparation and cleaning
- Too many milkers still don't wear gloves at milking

2011 Results

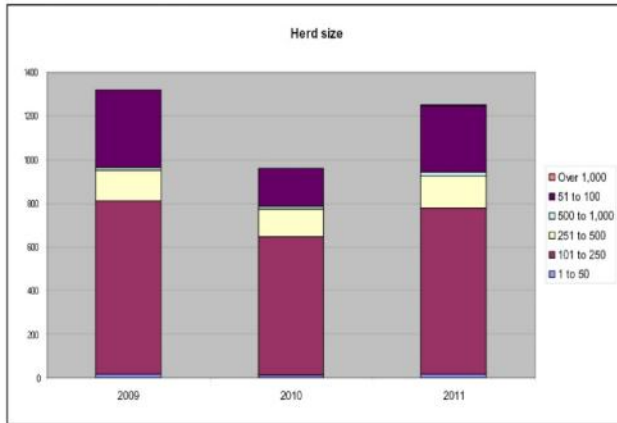
Regions and respondents



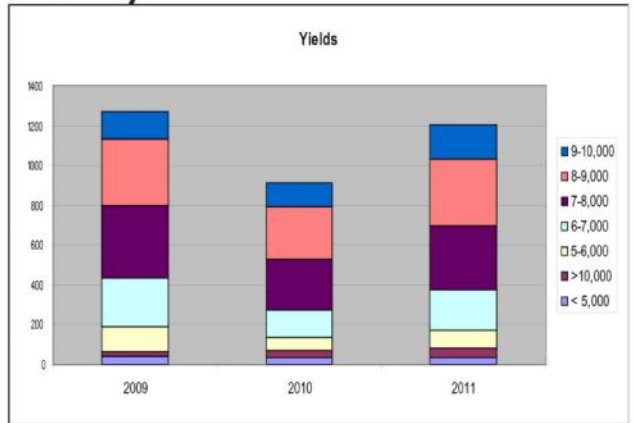
National Mastitis Survey Results

2011 Results

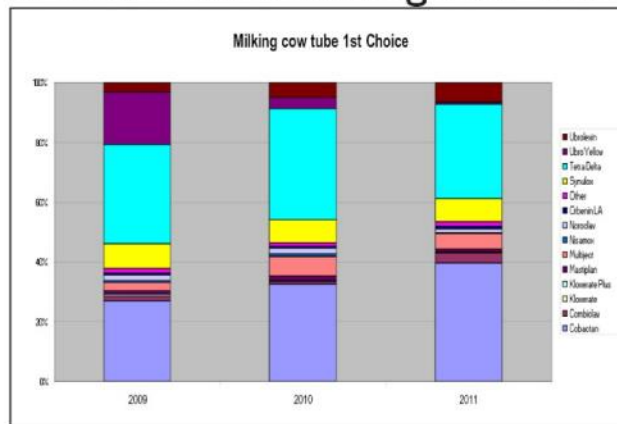
Herd size



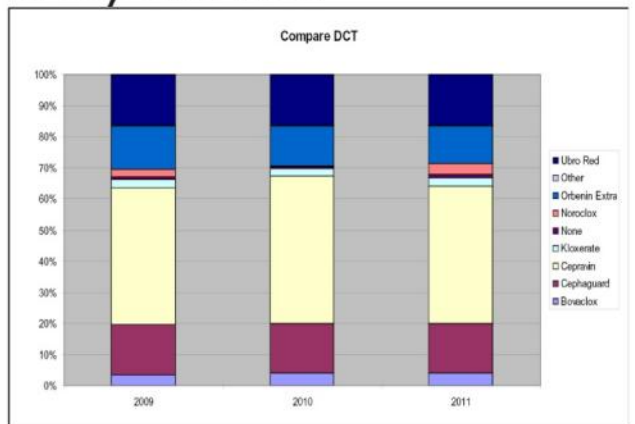
Herd yields



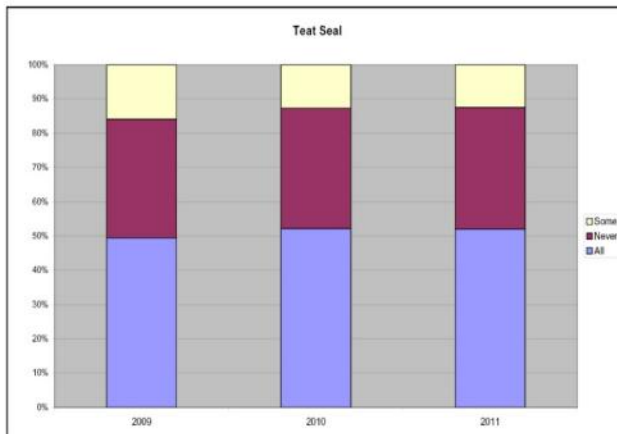
% first choice milking cow tube



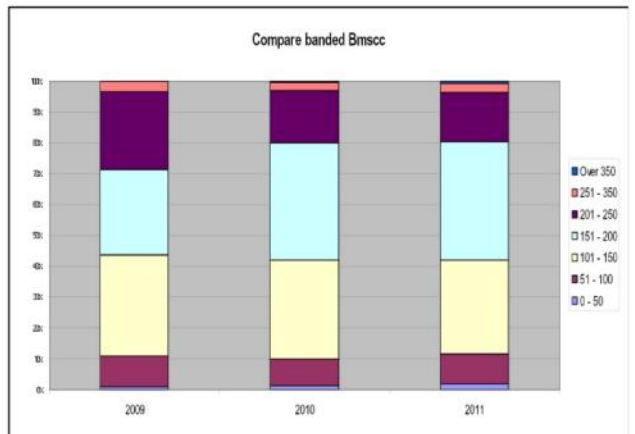
% dry cow tube



% teat seal



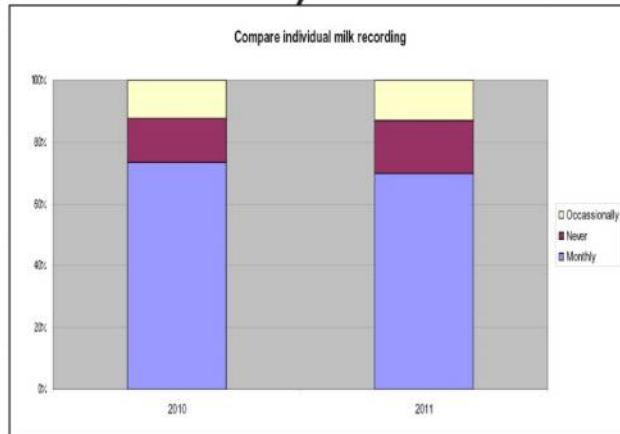
% banded BMSCC



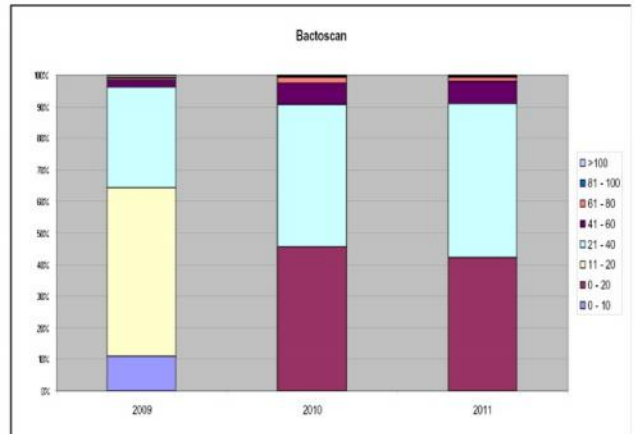
National Mastitis Survey Results

2011 Results

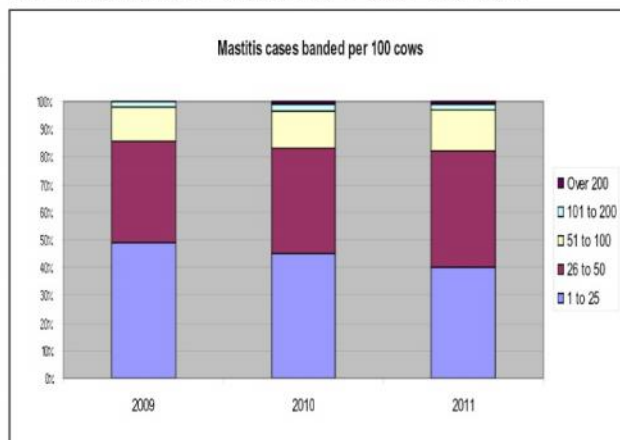
% who monthly milk record



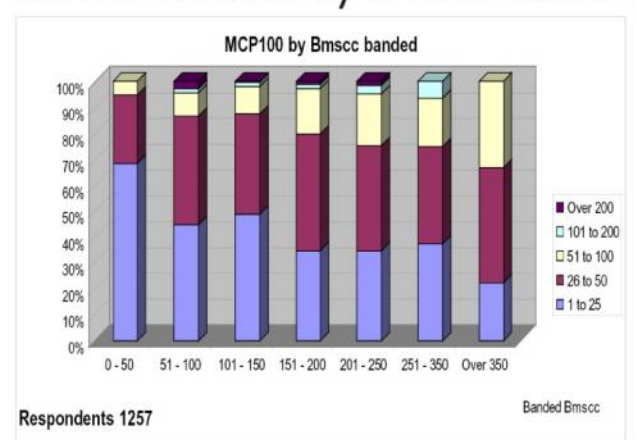
% bactoscan banded



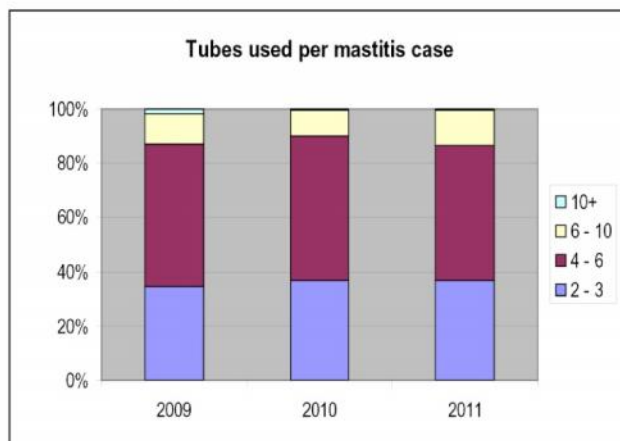
% mastitis cases/100 cows



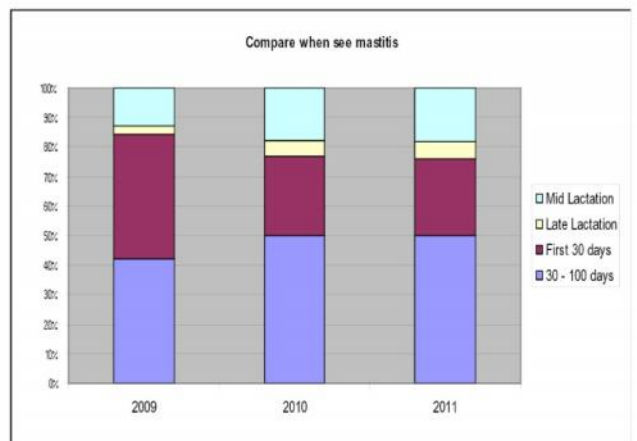
% MCP/100 cows by BMSCC banded



% tubes per clinical case



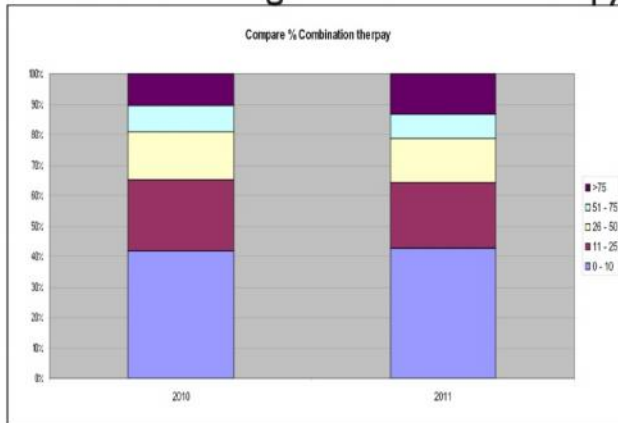
% when in lactation most mastitis



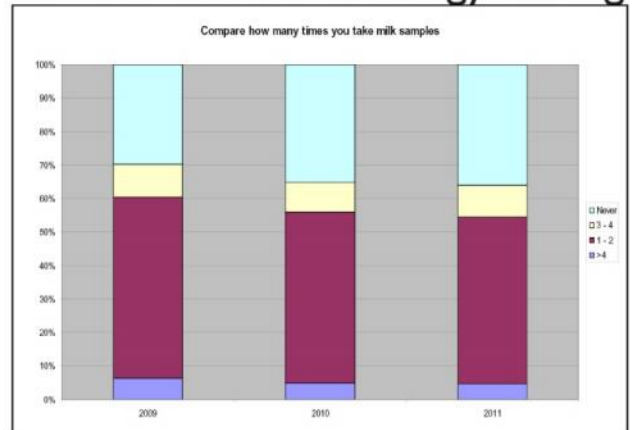
National Mastitis Survey Results

2011 Results

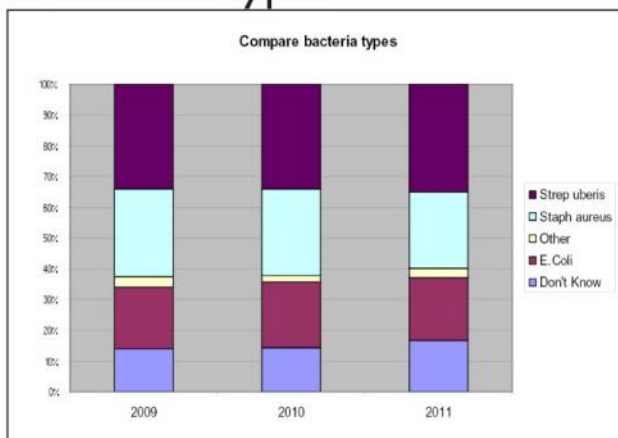
% cases receiving combination therapy



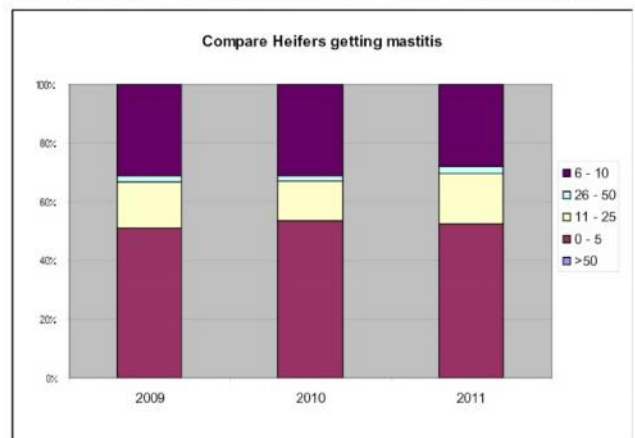
% how often bacteriology testing



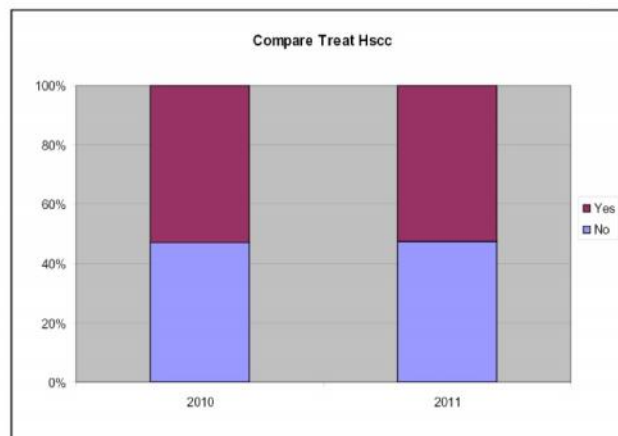
% bacteria types



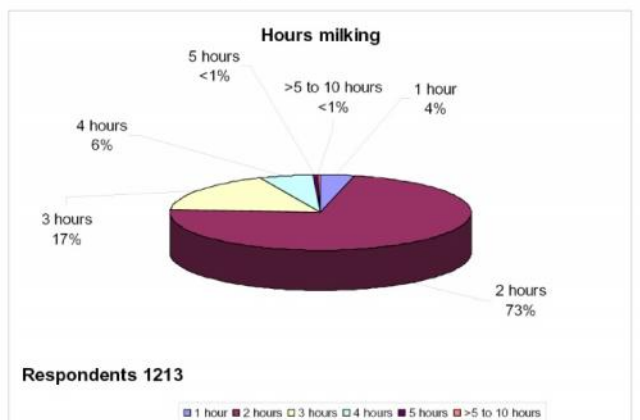
% heifer mastitis in 1st lactation



% treat HscC cows with no clinical signs



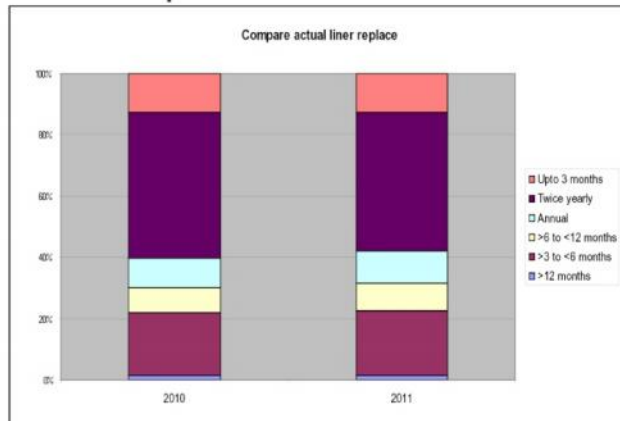
Hours milking - new for 2011



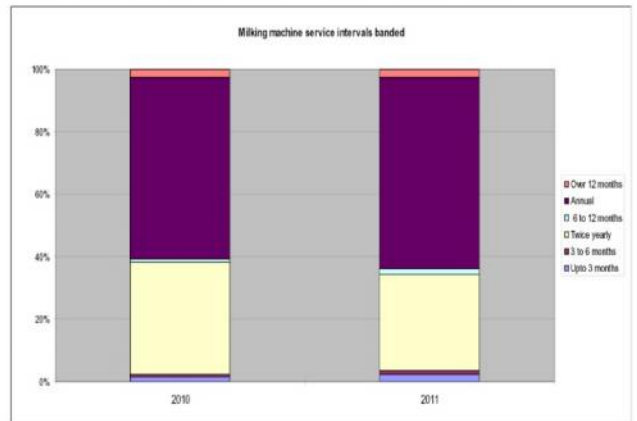
National Mastitis Survey Results

2011 Results

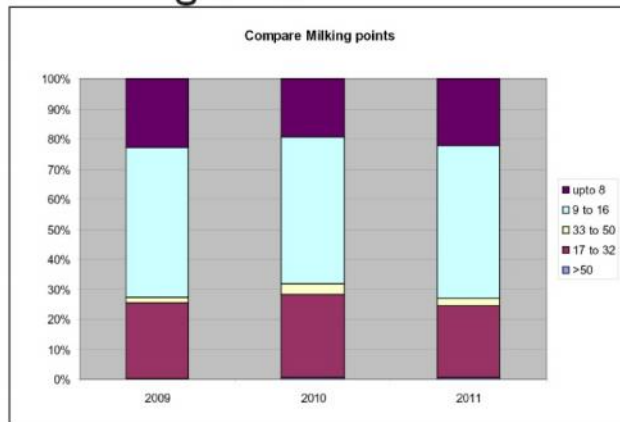
% liner replacement interval



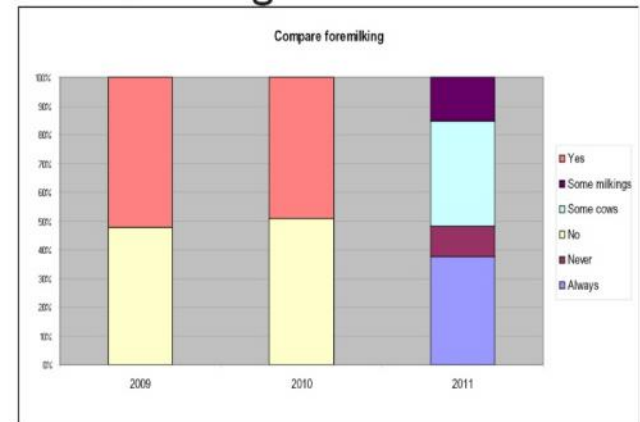
% machine service interval



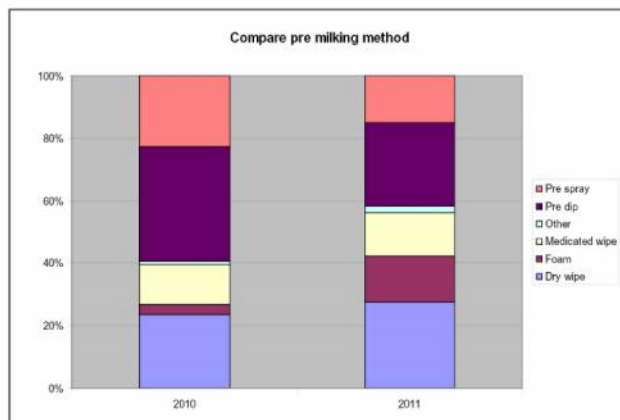
% milking units



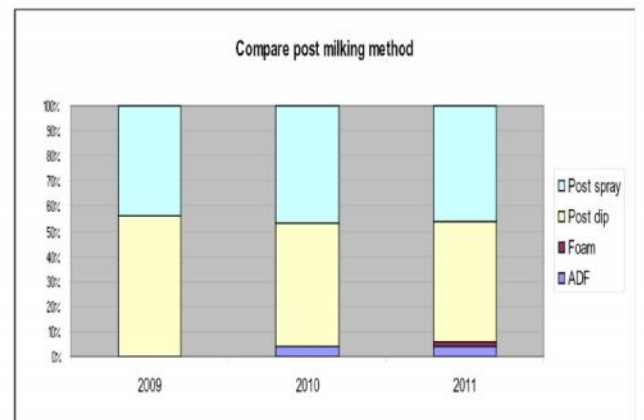
% foremilk



% pre-milking teat preparation method



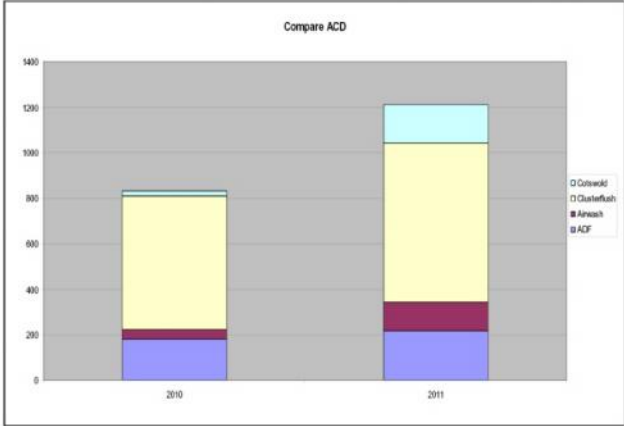
% post-milking teat disinfection method



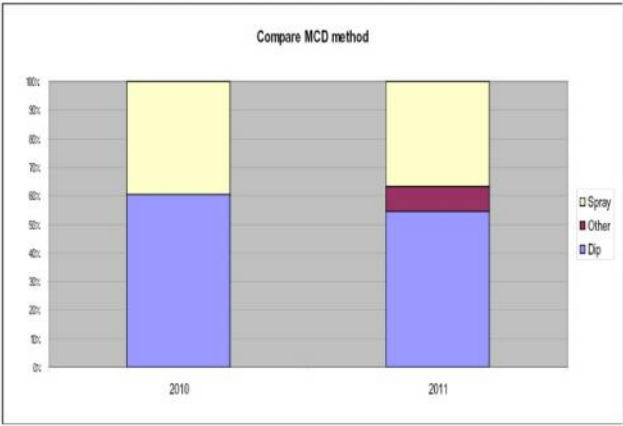
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2011 Results

2010 & 2011 automatic cluster disinfection



2010 & 2011 manual cluster disinfection



PLAIN END DIVIDER HERE