

2018

BRITISH MASTITIS CONFERENCE

Organised by

The Dairy Group



Topics:

- Reducing antimicrobial use
- Selective dry cow therapy at quarter level
- Research updates
- Mastitis in Africa
- The role of teat preparation & disinfectants
- Mastitis case study

Wednesday 7th November 2018

**Ricoh Lounge, Worcester Rugby Club,
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Worcester, Worcestershire WR3 8ZE**

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GENERAL INFORMATION

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CHAIRMAN'S INTRODUCTION

Welcome to the 2018 British Mastitis Conference.

The Organising Committee has worked hard since last year's conference to bring together a group of speakers, both international and home grown, that we believe will prove thought provoking and stimulating presentations. We have strived to balance the latest research with practical presentations and clear take home messages.

Our first paper looks at strategies for reducing antimicrobial use on dairy farms. This is followed by a paper on the very latest results on selective dry cow therapy at the quarter level.

Building on previous success and a session always endorsed by delegates, we have selected four posters from the Knowledge Transfer section for oral presentation. The four papers are followed by an opportunity for delegates to debate with the presenters.

After lunch, we will turn our attention away from the UK with a review of mastitis in developing Africa. This will be followed by a paper on the impact of teat preparation, teat disinfectants and teat hygiene on udder health. The conference will be closed, as has become the recent custom, with a practical mastitis control case study.

This year sees another excellent selection of high-quality poster submissions – covering a wide range of areas affecting udder health. I would urge you all to make time to review the posters and speak with the authors. Each year the presenters put a great deal of effort into providing the abstracts and preparing and presenting their posters.

We endeavour to find you the best speakers with the most relevant (and latest) information. This is only achievable thanks to all our generous sponsors. This year our sponsors are: Vetoquinol (Platinum), MSD Animal Health (Platinum & Scientific Poster Competition), Hipra (Gold), milkrite | InterPuls (Silver), Kilco (Silver), Boehringer Ingelheim (Silver), Norbrook (Bronze), Ambic (Bronze) and CID Lines (Bronze).

As always, the event could not happen without able administration, provided by Karen Hobbs and Anne Sealey at *The Dairy Group*.

Finally, thank you for attending and supporting the conference. I trust you will have an enjoyable and worthwhile day and we hope to see you at our 31stBMC in 2019.



Ian Ohnstad, British Mastitis Conference Chairperson
The Dairy Group

TIMETABLE of EVENTS

09:00	ARRIVE / REGISTRATION / COFFEE and POSTER DISPLAY	
09:45	CHAIRMAN'S INTRODUCTION	Ian Ohnstad The Dairy Group, UK
	Session One	Brian Pocknee DHC, UK
09:55	Strategies for reducing antimicrobial use.	Philip Elkin Westpoint Farm Vets, UK
10:25	Selective dry cow therapy at quarter level.	Andrew Bradley QMMS, UK
10:55	Questions and Discussion	
11:10	COFFEE and POSTERS	
	Research updates (also presented as posters)	Elizabeth Berry BCVA, UK
11:40	Effect of teatcup removal settings on milking efficiency and milk quality in a pasture based automatic milking system.	Pablo Silva Bolona University of Wisconsin, USA
12:00	Do herd mastitis patterns change over time?	Katharine Leach QMMS, UK
12:20	Quality-assessment of <i>E. coli</i> diagnostics in Danish veterinary clinics..	Michael Farre SEGES, Denmark
12:40	Teat disinfection: comparison of teat coverage with post milking teat disinfectant using a dip cup, vacuum operated hand-held teat sprayer and a platform mounted automatic teat disinfectant system.	Brian Pocknee Dairy Husbandry Consultancy, UK
13:00	LUNCH and POSTERS	
14:10	WELCOME BACK AND VOTING ON POSTERS	
	Session Three	Brian Pocknee DHC, UK
14.15	Mastitis in developing Africa.	Peter Edmondson Udderwise Ltd, UK
14.45	Meeting expectations with the teat disinfectants of today.	Mario Lopez DeLaval, USA
15.15	AHDB mastitis case study: Reducing clinical mastitis rate and antibiotic use in a Somerset dairy herd.	Rachel Hayton Synergy Farm Health, UK Daniel Macey Underhill Farm, Staple Fitzpaine, UK
15.45	Questions and Discussion	
16:00	POSTER AWARD and CLOSE	
16:05	TEA and DEPART	

Titles of Papers and Presenters

Scientific programme

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Selective dry cow therapy at quarter level. Andrew Bradley, QMMS, UK	9 – 20

Research Update Session (also presented as posters)

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Do herd mastitis patterns change over time? Katharine Leach, QMMS, UK	23 – 24
Quality-assessment of <i>E. coli</i> diagnostics in Danish veterinary clinics Michael Farre, SEGES, Denmark	25 – 26
Teat disinfection: comparison of teat coverage with post milking teat disinfectant using a dip cup, vacuum operated hand-held teat sprayer and a platform mounted automatic teat disinfectant system Brian Pocknee, Dairy Husbandry Consultancy, UK	27 – 28

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Mastitis in developing Africa Peter Edmondson, Udderwise Ltd, UK	29 – 32
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AHDB mastitis case study: Reducing clinical mastitis rate and antibiotic use in a Somerset dairy herd Rachel Hayton, Synergy Farm Health, UK Daniel Macey, Underhill Farm, Staple Fitzpaine, UK	43 - 53

Titles of Posters and Authors

Poster abstracts – presented at the Technology Transfer Session (presenting author underlined):

Internet of things enabled early detection of mastitis

C Davison¹, C Michie¹, I Andonovic¹, C Tachtatzis¹, M Gilroy², D McDonald² & J R Baines³ 55 - 56

¹Department Electronic and Electrical Engineering, University of Strathclyde, UK;

²Afimilk Ltd., Glasgow, UK; ³Fullwood Ltd, Ellesmere, UK.

Effectiveness of teat coverage with post milking teat disinfectant applied with a dip cup

Ian C Ohnstad¹, David Donaldson¹, Colin Kingston², Richard Hiley² and Brian R Pocknee¹ 57 – 58

¹ The Dairy Group, Taunton, UK; ² Ambic Equipment Ltd, Witney, UK

Machine learning predictions of herd mastitis diagnosis

Robert Hyde¹, Andrew Bradley^{1,2}, James Breen^{1,2}, Peter Down¹, Chris Hudson¹, Martin Green¹ 59 - 60

¹University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington Campus, UK; ²Quality Milk Management Services, Easton, UK

Maximising milking efficiency: A pilot study of current UK parameters and factors affecting the milking process

Tom Greenham & Dan Humphries 61 - 62

Advance Milking, Baschurch, UK

The role of Zinpro Availa@mins in mammary gland health; A research summary

Hugh R McConochie, Geiger, A., Gomez, A. & Rapp, C. 63 - 64

Zinpro Animal Nutrition, Inc., 5831 PJ Boxmeer, The Netherlands

Milk quality improvement initiative on Jersey

Ian C Ohnstad¹, Magdalena Drabble², Thierry Robine², and Andrew Bradley³ 65

¹The Dairy Group, Taunton, UK; ²Jersey Dairy, Trinity, Jersey; ³Quality Milk Management Services Ltd., Easton, UK

Udder health parameters from UK sentinel herds for 2017

J.E Breen^{1,2}, K.A. Leach¹, M.J. Green² and A.J. Bradley^{1,2} 67 - 68

¹Quality Milk Management Services Ltd, Easton, UK; ²School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK

Cow milk biorepository: a tool to investigate clinical and pre-clinical diseases under controlled conditions

Cristian Piras¹, Christopher K Reynolds², Barney Jones², Nick Taylor³, Michael R. Morris⁴, Rainer Cramer¹ 69 - 70

¹Department of Chemistry, University of Reading, UK; ²Centre for Dairy Research, School of Agriculture, Policy and Development, University of Reading, UK; ³University of Reading, UK; ⁴Waters Corporation, UK

Evaluation of selective culture-based media for detection and differentiation of bacteria associated with bovine clinical mastitis

F. Malcata¹, L. Viora¹, R. N. Zadoks^{1,2} 71 - 72

¹Scottish Centre for Production Animal Health and Food Safety, School of Veterinary Medicine, University of Glasgow, UK; ²Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, UK

Poster abstracts – also as an oral presentation in the Research Updates session

(presenting author underlined)

Effect of teatcup removal settings on milking efficiency and milk quality in a pasture based automatic milking system

Pablo Silva Bolona¹, D.J. Reinemann², and J. Upton³

¹Department of Dairy Science, University of Wisconsin-Madison, USA; ²Biological Systems Engineering Dept., University of Wisconsin-Madison, Madison, WI, USA;

³Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Ireland

21– 22

Do herd mastitis patterns change over time?

K.A. Leach¹, J.E Breen^{1,2}, M.J. Green² and A.J. Bradley^{1,2}

¹Quality Milk Management Services Ltd., Easton, UK; ²School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK

23 – 24

Quality-assessment of *E. coli* diagnostics in Danish veterinary clinics

Michael Farre¹, Henrik L. Martin¹, Betina B. Tvistholm¹, Jehan Ettema² and Lærke B. Astrup³

¹SEGES, 8200 Aarhus N, Denmark; ²SimHerd. 8800 Viborg, Denmark; ³Center for Diagnostics, Danish Technical University, Lyngby, Denmark

25 – 26

Teat disinfection: comparison of teat coverage with post milking teat disinfectant using a dip cup, vacuum operated hand-held teat sprayer and a platform mounted automatic teat disinfectant system

Ian C Ohnstad¹, Colin J Kingston², Richard J Hiley² and Brian R Pocknee¹

¹The Dairy Group, Taunton, UK; ²Ambic Equipment Ltd, Witney, UK.

27 – 28

FURTHER INFORMATION

Organised by *The Dairy Group*, BCVA, QMMS
and University of Nottingham

The Dairy Group



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Nottingham

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Organising Committee

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A global organization for mastitis control and milk quality

National Mastitis Council is a professional organization that promotes research and provides information to the dairy industry to help reduce mastitis and enhance milk quality. For more than 50 years, 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 and brochures
- 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 and workshops, providing educational opportunities for all segments of the dairy industry
- Funds the NMC Scholars program

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 veterinarians, milk quality consultants, dairy producers, university researchers and extension specialists, milk procurement field staff, equipment and supply representatives, regulatory 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 among 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, which contain all of the papers and posters presented at the meetings
- The NMC electronic newsletter addresses 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, udder health and mastitis prevention, control and treatment

Working together

Since 1961, NMC has coordinated research and educational 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

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STRATEGIES FOR REDUCING ANTIMICROBIAL USE

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SUMMARY

There is increasing pressure on agriculture in general, and the dairy sector specifically to reduce the use of antimicrobials. Mastitis treatment and prevention represents the most numerically significant use of antimicrobials in the sector and as such, steps to reduce the use of antimicrobials for mastitis should be investigated and utilised. Antimicrobial use should also be rational and appropriate, which MAY lead to an increase in antimicrobial use depending on which metric is used.

The primary route to reduction of antimicrobial use in mastitis is reduction of the rate of mastitis. Identifying the causal mastitis pattern, and then taking appropriate steps to reduce mastitis rate will often see the best return on investment. Other approaches which have attracted recent interest include selective dry cow therapy, and the use of rapid diagnostics and pathogen-based treatment protocols. These may have a role on many farms but care is needed around their implementation.

INTRODUCTION

Antimicrobial resistance is a global concern with the World Health Organisation publishing a report on methods to tackle the development of resistance in 2015 (28). In response, the UK government published a report calling for a reduction in the use of antimicrobials in animals, together with a national target for agriculture of 50 mg/kg by 2018. The Responsible Use of Medicines in Agriculture was tasked with and has produced sector specific targets (23). With specific regards to the dairy sector, they acknowledged the lack of interrogable, reliable data both on farm and off farm.

1 of the 4 specific focus areas for dairy, and 4 of the 6 Dairy Sector Targets relate to mastitis and the use of antimicrobials. It also highlighted the requirement for a collaborative approach involving vets, farmers and consultants.

Given the external pressure on the use of antimicrobials in mastitis, it is important to look at where those medicines are used for mastitis and look to either reduce the use, or where reduction is not justified, ensure all use is rational. This is an area of increasing interest for the veterinary and farming professions. A holistic approach involving quantifying current use and implementing appropriate strategies will see both reduced and rationalise use of antimicrobials for mastitis.

MEASURING USE

Antimicrobial use can be measured, and therefore monitored and benchmarked in a number of different metrics. Each of these has its own strengths and pitfalls and exist of a numerator and a denominator, for example milligrams of active substance (numerator) per kilogramme of animal at risk over a year (denominator). These can be based on either medicine purchases, or farm treatment records. Both of these have been shown to contain errors in under-reporting and misreporting (10). A comparison of the different metrics available showed them all to be relevant to certain circumstances but all represent issue with calculations and relevance to specific farms (17). The key when selecting a metric with which to benchmark is to be consistent, both in terms of the metric selected and its calculation, both within a farm and between farms.

The three main metrics used for benchmarking and comparing sales to farms that do not require the use of farm-specific weights are milligrams of active substance per population corrected unit of weight of animal (mg/PCU), number of standard daily treatments per animal (DDDVet), and the number of standard courses per animal (DCDVet). These utilise standardised animal weights and license sheet treatment recommendations to negate the requirement of individual treatment records to produce data which is comparable at a national level (9). These three parameters can be utilised in benchmarking antimicrobial usage as a part of active herd health planning (8).

It is worth noting that some of the antimicrobials under particular political pressure, such as those designated High Priority – Critically Important Antimicrobials, have a lower dose rate in terms of milligrams per kilogram treated. As such moving from these products to alternatives may lead to an increase in total use where that metric is used.

Numerous studies have shown mastitis to represent the most frequent antibiotic treatment of dairy cows (19, 24). As shown in table 1, when considering route of action, those medicines specifically indicated for intra-mammary administration represent a low proportion of the overall use on farms in mg/PCU, but a far greater proportion when considering number of courses of treatment (DCDVet).

Table 1: Mean influence of intramammary antibiotics on total use at a farm level. Data courtesy of Kingshay.

	Total	Intramammary	Lactating Cow Therapy	Dry Cow Therapy	% Intramammary
Mg/PCU	21.5	3.1	1.6	1.5	14.4
DCDVet	1.93	1.33	0.83	0.49	68.9

When looking at the distribution of number of courses of treatment with either lactating cow (LCT) or dry cow (DCT) intramammary treatment, as demonstrated in table 2, it is clear to see there is a wide range of values between farms, and significant improvements should be targeted for a number of farms.

Table 2: Distribution of DCDVet values for sales of lactating cow and dry cow intramammary antibiotics across 150 farms. Data courtesy of Kingshay

	Minimum	1 st Quartile	Median	3 rd Quartile	Maximum
LCT DCDVet	0.00	0.370	0.622	1.093	3.384
DCT DCDVet	0.00	0.229	0.516	0.688	1.093

Note, as these are annualised sales figures, it is possible due to buying patterns to see figures representing over 1 dry off per cow average.

REDUCING MASTITIS

In many cases, the simplest way to reduce the use of antimicrobials for mastitis is to reduce the rate of mastitis. This is as equally true as it is flippant. A structured approach to mastitis investigations and control has been shown to lead to significant improvements in mastitis rates of around 20% (12). This is mirrored by the benefits seen from appropriate implementation of herd health plans (15). However, it is important to be aware that the advisor's prior beliefs will have an influence on how likely they believe these benefits to be realised (11).

A routine approach to mastitis investigations would be based on a logical approach:

- Assess mastitis and somatic cell count records
- Strategic bacteriology
- Achieve a primary 'diagnosis'
 - Environmental vs contagious
 - Lactating period vs dry period
- Assess risk factors based on available evidence
- Agree interventions
- Review

(Adapted from 4)

This approach of diagnosis, assessing risk factors and agreeing interventions is a reliable approach for most herd health and disease control investigations.

There is a wide range of potential changes that can be implemented on farm that is beyond the realms of this paper, or indeed anything short of a bookshelf.

REDUCING IMPACT

Selective Dry Cow Therapy

Whole herd antibiotic dry cow therapy has been one of the mainstays of mastitis treatment and prevention since the middle of the last century (18), and as recently as 2002, was shown to have positive benefits in terms of clinical mastitis during and after the dry period, with the predominant causal bacteria prevented/treated being *Streptococcus uberis* (3).

More recently, the development and use of a non-antibiotic teat seal at the time of drying off has been shown to reduce the incidence of clinical mastitis before and after the dry period when used in isolation in cows with no history of clinical mastitis or raised somatic cell counts throughout this lactation (2). The product was also shown to be as efficacious as antibiotic dry cow therapy at preventing mastitis (13). This has led to interest and research into the applications of selective dry cow therapy on farm.

Studies have shown that various cut-offs can be used for selecting cows to receive antibiotics in association with a teat seal at drying off, or a teat seal alone, with these cut-offs acting as a proxy for whether the cow is infected or not. The California Mastitis Test has been shown to have relatively low sensitivity and therefore is likely to lead to a high proportion of animals with infections not treated and therefore not benefitting from the health benefits of appropriate dry cow therapy (21).

Using somatic cell count and clinical mastitis history to determine cut-offs has shown that economic savings can be made, as well as significant reductions in antimicrobial use. The most economically advantageous protocol will see a reduction of antibiotics of around a third, whereas some other protocols will see much greater reductions, but at the expense of increased disease (25).

It is important to remember though, when discussing selective dry cow therapy, that as advisors we are not the ultimate decision makers and the farmer's attitude is important. It has been shown that there are 4 factors that influence farmer's uptake of selective dry cow therapy:

- Financial consequences
- Uncertainty about consequences of withholding antibiotics
- Usage of teat seals
- Concerns around the potential negative consequences of selective dry cow therapy (26)

Given the above, the author's approach is to work with a farmer to set appropriate thresholds that are both likely to be beneficial in terms of economics without being detrimental to cow health, but also in line with the farmer's attitude to risk and the above factors. In practice this means that a farm's individual cut-offs for application of selective dry cow therapy will be particular to themselves and fluid over time as their confidence with the

principle and application changes. It is important to remember that any cow whose teats cannot be appropriately sterilised prior to infusion may benefit from application of an antibiotic therapy prior to sealing. This includes severe teat end damage, or stretched supportive udder ligaments leading to low clearance from the ground.

The Kingshay Antimicrobial database shows that selective dry cow therapy is already widely practised, with 75% of herds withholding antibiotics from at least 20% of cows.

In the author's opinion, the conversations around selective dry cow therapy offers an opportunity to ensure that when antibiotics are used at drying off, they are appropriate for the task. Bacterial infections may be categorised on many grounds, including chronicity. Somatic cell count patterns can be suggestive of particular pathogen with gram positive infections more likely to lead to longer term rises of somatic cell count compared with gram negative or no growth infections (5). As such, cows can be differentiated into three categories based on somatic cell count and clinical mastitis history – those likely to be uninfected and should receive no antibiotics, those where infection status is uncertain and should receive short-acting, broad spectrum antibiotics, and those likely to be infected with a gram-positive bacteria and should receive a narrow-spectrum antibiotic.

Culture-based treatment protocols

There has been recent interest in the use of rapid diagnostics on farm or at a clinic to influence treatment protocols. Much of the original work on this was based on delaying treatment (other than non-steroidal anti-inflammatories) for cows with no systemic involvement until the results of the diagnostics were complete, and then using antibiotics for gram positive infections only. These studies showed 56% of cases receiving no antibiotics, with a tendency towards reduced days of milk discard, and no significant negative health effects (14).

However, when considering the delay in treatment effect on gram positive cases, and the potential farm variation in relative importance of gram-positive infections, the approach is not universally applicable. The cost-effectiveness and potential impact on cow health indicate that this approach may not be suitable where gram positive bacteria account for over 20% of clinical mastitis cases (6).

The most commonly used rapid-diagnostic kit in the UK (Veto-rapid, Vetoquinol) has been compared against both standard bacteriology (27) and either standard bacteriology or PCR (7). Both studies found poor negative and positive predictive values when diagnosing specific bacteria, but adequate sensitivity, specificity, positive predictive value and negative predictive value when using the kit to determine whether the mastitis was caused by gram positive bacteria, or gram negative bacteria/no growth. However, the presence

of pathogens which are not identifiable by this technique such as *Mycoplasma bovis*, significantly reduce performance.

The rapid diagnostic-based treatment culture approach detailed above may be improved in the author's opinion by treating initially with a single treatment of broad spectrum antibiotics before adapting the treatment protocol based on the presence or absence of gram positive bacteria. This will reduce the benefits of reduced antimicrobials but potentially benefit cure rates and be more applicable for herds with over 20% of clinical mastitis attributable to gram positive bacteria. This approach requires further investigation.

Systemic treatment

The use of parenteral antibiotics to treat certain cases of clinical mastitis, predominantly those caused by *Staphylococcus aureus*, has been shown historically to have a beneficial effect (20). However, other studies show no benefit to adding systemic treatment with antibiotics to intramammary treatment, and on balance the requirement for higher doses compared with minimal benefits, means that systemic treatment is often not warranted (1).

Systemic antibiotics have been shown to be of no benefit for mastitis caused by *Escherichia coli* (22), although parenteral penicillin has been shown to be beneficial for outcomes in *Streptococcus uberis* mastitis (16).

Given that there is conflicting evidence on the benefits of parenteral administration of antibiotics on the outcomes of clinical mastitis, its use must be put into question and justified on a case by case basis.

CONCLUSIONS

Mastitis represents a significant use of antimicrobials on dairy farms. Measuring, monitoring and benchmarking use is important to set a baseline for tracking performance. Antimicrobial use can be reduced through reducing mastitis case rate or reducing the impact of mastitis on use through selective dry cow therapy, rapid diagnostics or ensuring use is justified.

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NOTES

SELECTIVE DRY COW THERAPY AT QUARTER LEVEL

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SUMMARY

The importance of the dry period in mastitis epidemiology is well acknowledged, as is the role antibiotic dry cow therapy can play at this time. However, pressure on the use of antimicrobials in food producing animals, and prophylactic use in particular, has brought the use of antibiotic dry cow therapy into focus. Whilst the selective use of antibiotic dry cow therapy at the cow level is now well established, the selective use at quarter level is less well understood. This paper outlines the preliminary findings of a large UK study investigating selection of antibiotic treatment at the quarter level in both low and high SCC cows at drying off using the California Mastitis Test (based on its widespread availability and low cost). Preliminary analysis of data from this study suggests that in herds such as the type recruited to this study (ie low SCC and low prevalence of contagious pathogens) there is probably no justification for the general use of supplementary antibiotics in CMT positive quarters in low SCC cows at drying off. However, there may be scope to further reduce antibiotic use by withdrawing antibiotics from low SCC (CMT negative) quarters in high SCC cows. Any such approach should be implemented with care and only when a mechanism for monitoring the likely impact is in place.

INTRODUCTION

The importance of the dry period in mastitis epidemiology is well acknowledged (3) as are the benefits of the use of antibiotic dry cow therapy. The selective use of antibiotic dry cow therapy in combination with blanket use of an internal sealant has been advocated for a number of years and is well supported in the scientific literature (6,9) and by UK based research (2, 4,5,10). Concern around the prophylactic use of antibiotics has resulted in questions being raised about the use of antibiotics in quarters not infected at drying off, primarily from the perspective of reducing antibiotic use (11). Historically, cow level application of antibiotic dry cow therapy has been advocated, primarily because quarters are not independent within cows and therefore an increased risk of infection has been perceived in 'uninfected' quarters in 'infected' cows (1). However, there is evidence that this lack of independence is less marked with 'environmental' than 'contagious' mastitis pathogens. There has also been concern that the 'cow level' approach will result in some infected quarters not being treated and therefore being at increased risk of being infected at calving. Ultimately, a quarter level selective

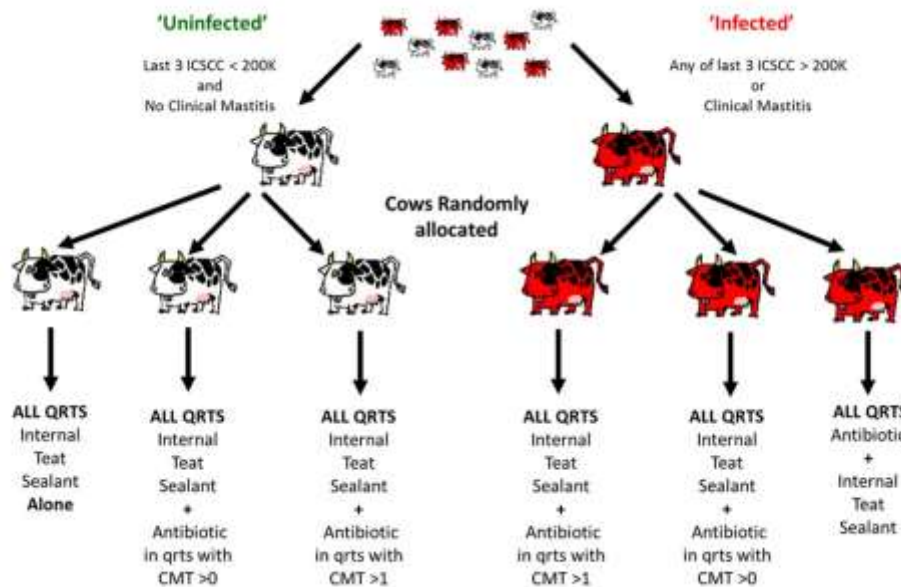
dry cow treatment approach may potentially further reduce the use of antibiotics when compared to selective dry cow treatment at the cow level and could result in better overall dry period outcomes. However, to date, peer-reviewed data on the outcome of dry period treatments allocated at quarter level is very limited. In an Australian study (7) restricted to cows infected at drying off, which suggested increased new infections following quarter level treatment of cows infected at drying off, the effect was dominated by *S. uberis* and *S. aureus*. These findings, and those of a following study (8), may not be transferable to herds with low somatic cell count, therefore further data is needed to inform the UK situation. This paper reports preliminary results from the first large study to investigate the outcome of quarter level dry cow treatments in low SCC herds, with low levels of contagious mastitis, in the UK.

MATERIALS AND METHODS

Commercial farms in the south-west of England were selected to participate on the basis of 1) likely compliance with the study protocol, 2) a bulk milk somatic cell count typically less than 200,000 cells/ml 3) monthly individual cow somatic cell count testing and 4) retrospective records of clinical mastitis for at least 12 months.

Cows, within herds, were stratified ('infected' or 'uninfected') using somatic cell count and clinical mastitis history, before being randomly allocated to one of three treatment groups: CLT, QLT0 and QLT1. The CLT (Cow Level Treatment) group were allocated, using somatic cell count and clinical mastitis history, into animals eligible for the use of an internal teat sealant alone (Cephalock™) or an internal teat sealant in combination with antibiotic dry cow therapy (CEFA-SAFE™) - importantly this decision was applied at the cow level with all quarters within a cow receiving the same treatment. Within the QLT0 (Quarter Level Treatment - CMT>0) and QLT1 (Quarter Level Treatment - CMT>1) groups, quarters within cows were allocated (based on a CMT score of >0 or >1 respectively) to receive an internal teat sealant alone (score below the threshold) or an internal teat sealant in combination with antibiotic dry cow therapy (score above the threshold) depending on the quarter California Mastitis Test (CMT) score at drying off. The overall design is illustrated in Figure 1 below. The quarter was the experimental unit. It was anticipated that approximately 250 cows would be recruited to each treatment group (750 cows, 3,000 quarters in total). Cows were recruited over a 12-month period to allow seasonal effects to be investigated.

Figure 1 An illustration of the study design



At Drying Off: Cows were recruited prior to their final milking in lactation, assessed for suitability for enrollment and randomly allocated to one of the three treatment groups. All quarters of all cows were subjected to the CMT, prior to being aseptically sampled. Samples for bacteriology and somatic cell count analysis were collected from each quarter. Data on parity, yield at drying off, historic somatic cell count data, clinical mastitis history, treatment history and other relevant clinical data were collated. Samples were maintained at or below 8°C whilst transported to the laboratory for analysis. Treatments were administered, following strict asepsis and according to datasheet recommendation.

At Calving: Within one week of calving, samples for bacteriology and SCC were collected from each quarter and a CMT test carried out on each quarter.

Post Calving: Between 7 and 14 days post calving, a CMT test was performed on each quarter and quarter milk samples were collected for SCC determination.

After Calving until 100 Days Post-Calving: Cows were managed according to normal husbandry practices on the farm. Any disease or concurrent treatments were recorded. Any cases of clinical mastitis were scored for severity and recorded by trained farm staff. Clinical samples were frozen before transport in batches to the laboratory.

Laboratory Methods

Microbiological investigation and Somatic Cell Counts were carried out in accordance with the methods recommended by the International Dairy Federation (IDF) (Bulletin No 132, 1981), International standard 13366-1: 1997 (E) and 13366-2: 1997 (G). In summary, samples were inoculated onto blood, MacConkey, and Edwards agar and incubated for 72 hours at 37°C. Both the blood and Edwards agar were inoculated with 10µl of milk. The MacConkey agar was inoculated with 100µl of milk to enhance the chances of isolation of *Enterobacteriaceae*. All organisms were identified and enumerated. Organisms were identified primarily by using MALDI-TOF MS, but also where necessary on the basis of typical colony morphology, gram staining, and further biochemical tests.

Assessment of Effectiveness

At the time of writing cow recruitment was complete, however some cows had not yet calved and outcomes were still being assessed. Four primary outcomes are being assessed as outlined below:

Outcome 1: Cure of Existing IMIs.

Bacteriological Cure: The overall, and species specific, cure rates were estimated and compared between groups. A cure is defined as the absence of a pathogen in the post calving sample that was present at drying off.

SCC Cure: Cure rates were also estimated and compared between groups, at both the cow and quarter level, by investigating SCC movements around pre-defined thresholds.

Outcome 2: Acquisition of New IMIs.

Bacteriological New IMI: The overall, and species specific, new infection rates will be estimated and compared between groups. A new infection is defined as the presence of a pathogen in the post calving sample that was not present at drying off.

Outcome 3: A Successful Dry Period Outcome.

Successful dry period outcomes will be estimated and compared between groups. A successful outcome will be defined in two ways; firstly, as the absence of a major pathogen from the post calving sample and secondly as the absence of any mastitis pathogen from the post calving sample.

Outcome 4: Prevention of Clinical Mastitis in the 1st 100 Days of the Subsequent Lactation.

The overall, and species specific, incidence rate of clinical mastitis will be assessed in the first 100 days of lactation and compared between groups.

In addition to the primary outcomes outlined above, a full exploration of the data will be undertaken, with the plan of better understanding the impact of quarter *vs* cow level selection of dry cow therapy treatments. This will include, but will not be limited to, the primary outcomes at the cow and quarter level

as well as the impact on overall antibiotic use during the dry period and in early lactation. Data will also be explored to better understand the suitability of CMT *vs* SCC for making quarter level decisions at drying off as well as the value of single *vs* repeat SCCs prior to dry off.

Statistical Analysis

Power and Sample Size: Calculations based on UK data suggested that assuming 80% power and 95% confidence in a two-sided test the sample sizes allow detection of a 6% (absolute) difference in a successful dry period outcome, given a baseline level of 70% of quarters being pathogen free post calving in the CLT group.

Data were collated and initially analysed using Excel and Access (Microsoft Corp) and Minitab (Minitab Inc). Descriptive and graphical analyses were carried out to explore the data. Univariable analysis of treatment efficacy was performed using the Chi-Square test to investigate differences in proportions between groups. Analysis was undertaken assessing ‘infected’ and ‘uninfected’ cows both separately and together.

RESULTS

A total of 807 cows from six relatively low SCC herds (typically less than 200,000 cells/ml) were recruited to the study, 401 were defined as ‘infected’ and 406 defined as ‘uninfected’ by historic SCC and clinical mastitis data. These cows were temporally matched and then randomly allocated to treatment group. Key characteristics of the treatment groups are summarised in Table 1.

Table 1 Key characteristics of the study groups

Infection Status at Dry Off	Infected			Uninfected		
Treatment Group	CLT	QLTO	QLT1	CLT	QLTO	QLT1
n	133	133	135	134	137	135
Parity (Mean)	2.56	2.7	2.48	2.01	2.10	2.10
Parity (Min)	1	1	1	1	1	1
Parity (Max)	8	7	7	8	6	6
Yield (l) (Mean)	16.7	16.6	17.0	18.7	18.1	18.5
Yield (l) (Min)	2.0	2.7	1.8	4.5	3.3	3.0
Yield (l) (Max)	46.6	39.4	43.7	47.7	36.0	40.0
SCC-1 (Median)	267	263	286	63	73	73
SCC-1 (Min)	13	14	23	9	9	12
SCC-1 (Max)	4,683	4,955	5,297	198	199	194
SCC-2 (Median)	201	199	220	46	57	56
SCC-2 (Min)	18	29	17	10	10	7
SCC-2 (Max)	2,850	9,098	6,048	175	190	198

Infection Status at Dry Off	Infected			Uninfected		
SCC-3 (Median)	190	211	174	47	49	38
SCC-3 (Min)	5	5	12	8	6	8
SCC-3 (Max)	6,221	1,845	3,934	193	198	185

(SCCs in the 3 months prior to drying off (,000 cells/ml))

The results of CMT tests conducted at drying off are summarised in Table 2, by infection status and treatment group. As might be expected, CMT scores were higher in the ‘infected’ category, with approximately 70% of quarters exhibiting at least a ‘trace’ reaction to the CMT, whilst approximately 70% of quarters in the ‘uninfected’ category demonstrated no reaction to the CMT.

Table 2 Proportion of quarters with each CMT score at drying off, by treatment group

Infection Status at Dry Off	Infected			Uninfected		
Treatment Group	CLT	QLT0	QLT1	CLT	QLT0	QLT1
n	532	532	540	536	548	540
Score 0	31.2	29.9	35.9	72.0	69.7	67.8
Score 1	27.1	26.3	18.5	19.4	20.3	19.3
Score 2	28.8	26.9	32.4	7.1	7.9	11.3
Score 3	13.1	16.9	13.2	1.5	2.2	1.7

The sensitivity and specificity of the CMT test result in excess of 0 for identifying quarters with an SCC>200,000 cells/ml at drying off was 0.86 and 0.73 in all cows (PPV 0.68, NPV 0.84), 0.63 and 0.79 in cows defined as ‘uninfected’ at drying off (PPV 0.46, NPV 0.88) and 0.87 and 0.61 in cows defined as ‘infected’ at drying off (PPV 0.77, NPV 0.76). The sensitivity and specificity of the CMT test result in excess of 1 for identifying quarters with an SCC>200,000 cells/ml at drying off was 0.56 and 0.93 in all cows (PPV 0.85, NPV 0.75), 0.33 and 0.96 in cows defined as ‘uninfected’ at drying off (PPV 0.69, NPV 0.83) and 0.65 and 0.88 in cows defined as ‘infected’ at drying off (PPV 0.89, NPV 0.63).

The prevalence of ‘infection’ at dry off for some of the key mastitis pathogens is summarised in Table 3. The overall prevalence was low with only 10.1% of quarters culturing a major pathogen. Minor pathogens were the most common finding with 50.0% of quarters culturing positive for one or more minor mastitis pathogens.

Table 3 Summary of key bacteriological findings in quarters at drying off.

Cow Level 'Infection' Status at Dry Off *	Overall (n = 3,228)		Infected (n =1,604)		Uninfected (n=1,624)	
Pathogen	n	%	n	%	n	%
<i>Staphylococcus aureus</i>	29	0.90	19	1.18	10	0.62
<i>Streptococcus uberis</i>	29	0.90	25	1.56	4	0.25
<i>Streptococcus dysgalactiae</i>	7	0.22	7	0.44	0	0.00
<i>Escherichia coli</i>	14	0.43	7	0.44	7	0.43
<i>Enterobacteriaceae</i>	17	0.53	10	0.62	7	0.43
<i>Yeast spp</i>	21	0.65	12	0.75	9	0.55
All Major Pathogens	323	10.1	202	12.8	121	7.52
Major Gram-positive Pathogens	242	7.59	160	10.1	82	2.09
Major Gram-negative Pathogens	77	2.41	39	2.47	38	2.36
Minor Pathogens	1595	50.0	873	55.3	722	44.8
No Growth	1359	42.6	565	35.8	794	49.3

* as defined by historic SCC and clinical mastitis data

The sensitivity and specificity of the CMT test for identifying the presence of a major pathogen or a major Gram-positive pathogen at drying off is outlined in Table 4. As might be expected, the sensitivity and specificity varied between the cows with different infection categories at drying off, with the sensitivity being higher in the 'infected' category and specificity higher in the 'uninfected' category. The sensitivities and specificities of the different CMT thresholds were very similar when comparing their ability to detect any major pathogen or a Gram-positive major pathogen.

Table 4 The sensitivity and specificity of the CMT test at different thresholds for identifying 'infected' quarters at drying off

Cow Level 'Infection' Status at Dry Off *	Overall		Uninfected		Infected	
Test	Sens	Spec	Sens	Spec	Sens	Spec
Major Pathogen at Dry Off						
CMT > 0	0.64	0.53	0.31	0.70	0.83	0.35
CMT > 1	0.43	0.75	0.12	0.90	0.62	0.59
Major Gram-positive Pathogen at Dry Off						
CMT > 0	0.67	0.53	0.35	0.70	0.83	0.34
CMT > 1	0.49	0.75	0.15	0.90	0.67	0.59

* as defined by historic SCC and clinical mastitis data

At the time of writing, data was available from 2,952 quarters from 765 cows. The prevalence of 'infection' at calving for some of the key mastitis pathogens

is summarised in Table 5. The overall prevalence was low with only 11.9% of quarters culturing a major pathogen. Minor pathogens were the most common finding with 33.4% of quarters culturing positive for one or more minor mastitis pathogens. More quarters were free of any pathogen post calving than prior to drying off, though this difference was ‘driven’ by control of minor mastitis pathogens.

Table 5 Summary of key bacteriological findings in quarters at calving

Cow Level ‘Infection’ Status at Dry Off *	Overall (n = 2,952)		Infected (n =1,452)		Uninfected (n=1,500)	
Pathogen	n	%	n	%	n	%
<i>Staphylococcus aureus</i>	8	0.27	4	0.28	4	0.27
<i>Streptococcus uberis</i>	48	1.63	22	1.52	26	1.73
<i>Streptococcus dysgalactiae</i>	9	0.30	5	0.34	4	0.27
<i>Escherichia coli</i>	18	0.61	4	0.28	14	0.93
<i>Enterobacteriaceae</i>	41	1.39	17	1.17	24	1.60
<i>Yeast spp</i>	32	1.08	20	1.38	12	0.80
All Major Pathogens	352	11.9	193	13.3	159	10.6
Major Gram-positive Pathogens	222	7.52	122	8.40	100	6.67
Major Gram-negative Pathogens	116	3.93	59	4.06	57	3.80
Minor Pathogens	985	33.4	433	29.8	552	36.8
No Growth	1725	58.4	889	61.2	836	55.7

* as defined by historic SCC and clinical mastitis data

When univariable comparisons were made between treatment groups, within infection categories at drying off, quarters in cows in the ‘infected’ category at drying off, in the CLT group, were significantly more likely to be free of any pathogen post calving than quarters in cows in the QLT1 group (64.9% vs 56.8%; $p < 0.05$); The QLT0 group did not differ from either of the other treatment groups ($p > 0.05$). Quarters in cows in the ‘uninfected’ category at drying off, in the CLT group, were significantly less likely to be free of any pathogen post calving than quarters in cows in the QLT0 group (51.5% vs 60.3%; $p < 0.05$); the QLT1 group did not differ from either of the other treatment groups ($p > 0.05$).

These differences were not borne out in the ‘infected’ category at drying off when considering major pathogens. However, quarters in cows in the ‘infected’ category at drying off, in the CLT group, were significantly less likely to be free of a minor mastitis pathogen post calving than quarters in cows in the QLT1 group (27.2% vs 56.8%; $p = 0.05$); the QLT0 group did not differ from either of the other treatment groups ($p > 0.05$).

Similarly, the minor pathogen prevalence post calving, in quarters in cows in the ‘uninfected’ category at drying off, was significantly lower in the QLT0 group than in quarters in cows in the CLT group (33.7% vs 41.8%; $p < 0.05$); the QLT1 group did not differ from either of the other treatment groups ($p > 0.05$). Differences were evident in the prevalence of major pathogen post calving between the quarter level treatment groups in cows defined as ‘uninfected’ at drying off, with the prevalence of major pathogens being significantly higher in the QLT1 group compared to the QLT0 group (13.9% vs 8.3%; $p < 0.05$). Whilst the QLT1 group was not significantly different from the CLT group, there was a trend for a higher prevalence of major pathogens in the QLT1 group (13.9% vs 9.6%; $p = 0.07$).

Quarter SCCs measured between 7 and 14 days post calving are summarised in Table 6. Analysis revealed a significant difference between treatment groups within infection category. SCCs post calving were significantly higher, in quarters in cows defined as ‘infected’ at drying off, in the QLT1 group than either of the other treatment groups. SCCs post calving were significantly higher, in quarters in cows defined as ‘uninfected’ at drying off, in the CLT group than either of the other treatment groups.

Table 6 Summary of quarter somatic cell counts in samples collected 7-14 days post calving

Quarters in cows defined as infected at drying off				95% CI	
Treatment Group	n	Mean SCC (,000/ml)*	StDev	Lower	Upper
CLT	484	42 ^a	4.19	37	48
QLT0	467	45 ^a	4.20	39	51
QLT1	502	59 ^b	4.65	52	68
Quarters in cows defined as uninfected at drying off				95% CI	
Treatment Group	n	Mean SCC (,000/ml)*	StDev	Lower	Upper
CLT	482	63 ^a	5.39	55	72
QLT0	491	43 ^b	4.32	37	49
QLT1	499	46 ^b	4.33	40	53

^{a,b} superscripts within column, within infection category differ.

* analysis carried out on log transformed data; mean value re-transformed for reporting

Antibiotic use was assessed in each of the treatment groups with respect to the number of cures effected by treatment. In the cows defined as ‘infected’ at drying off, cow level treatment achieved the highest ‘cure’ rate of major pathogens (97.4%) but was associated with the highest level of antibiotic tube usages/cure (13.66 tubes/major pathogen cure. The number of tubes used per cure decreased with quarter level selection. In cows defined as ‘uninfected’ at drying off, the ‘self-cure’ was 100% in the CLT and QLT0 groups. Only three quarters failed to ‘cure’ in the QLT1 group, though it is

worthy of note that these apparent failures to cure were due to infection with *E. coli*, *Candida parapsilosis* and *Candida tropicalis*, against which antibiotic dry cow therapy is unlikely to be effective.

Table 7 Antibiotic use outcomes by treatment group

Treatment Group	n	Number of quarters 'infected' with a major pathogen at drying off	Number of quarters curing	Cure Rate (%)	Number of antibiotic tubes used	Number of antibiotic tubes used / major pathogen cure
Cows defined as infected at drying off						
CLT	519	39	38	97.4	519	13.66
QLT0	521	56	52	92.9	364	7.00
QLT1	517	49	44	89.8	234	5.32
Cows defined as uninfected at drying off						
CLT	530	22	22	100	0	0
QLT0	541	40	40	100	163	4.08
QLT1	531	23	20	87.0	69	3.45

DISCUSSION AND CONCLUSIONS

This study is the first large scale investigation into the selection of dry cow therapy at the quarter level in the UK. Historically, such approaches have not been favoured on the basis of the lack of independence of quarters within cows, meaning that it was considered that the risk of missing a major pathogen infection in another quarter of a high cell count cow was too high. For this reason, this study focussed on relatively low SCC herds likely to reflect the general population of herds currently in the UK.

The prevalence of infection at dry off in this study was low, with classic contagious pathogens such as *S. aureus* representing less than 10% of all major pathogen infections; less than 1% of quarters were apparently infected with this pathogen at drying off. The aetiology in these herds is clearly 'environmental' and minor pathogens represented the vast majority of infections present at drying off. Cure rates and apparent 'self-cure' rates in this study were very high with 'new infection' accounting for the majority of infections present at calving. Minor pathogens again predominated at calving.

The CMT would appear to be a cheap, rapid and viable, albeit imperfect, way of targeting infected quarters at drying off. When taking into account the differing sensitivity and specificity of this test in the different infection categories, the CMT arguably performs as required irrespective of the threshold adopted, with specificity higher in the 'uninfected' category and

sensitivity higher in the ‘infected’ category, reflecting the need for a higher level of confidence in identifying uninfected and infected quarters respectively.

Preliminary analysis suggests that the impact of selecting treatments at the quarter level appears to be different in the different infection categories. Overall the primary effect seems to be on SCC and minor pathogens, rather than major pathogens (probably reflecting the relative prevalence of the former). There appears to be little justification for superimposing antibiotic treatment on a teat sealant in low SCC cows at drying off, as self-cure rates appear to be very high and major pathogen prevalence is low – removal of minor pathogens and further SCC reduction is probably not sufficient justification alone. In addition, in herds such as the ones in this study, there appears to be little risk associated with the removal of antibiotic from very low SCC (CMT score 0) quarters in “infected” (high SCC) cows, as there is minimal impact on SCC post calving and little effect on apparent cure rates of major pathogens.

The effect of targeting antibiotics by identifying infected quarters in low SCC cows and uninfected quarters in high SCC cows has merit, but it would appear that the impact of such targeting is not always clear. In herds such as those in this study the risk of missing significant Gram-positive major pathogen infections would appear to be low, suggesting that this may be a viable approach. However, the farmer and practitioner alike should be aware that this data has been generated from a small number of low SCC herds with a low prevalence of contagious pathogens and as such the findings should be interpreted and applied in the light of farm specific circumstances.

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NOTES

EFFECT OF TEATCUP REMOVAL SETTINGS ON MILKING EFFICIENCY AND MILK QUALITY IN A PASTURE BASED AUTOMATIC MILKING SYSTEM

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Automatic milking systems are becoming an increasingly adopted technology by dairy farmers. It is thought that up to 50% of new milking installations in the European Union will be automatic milking systems (1). In these systems, a key factor for achieving high efficiency is to maximize the number of milkings performed by the robot in a day (2). One option for achieving this goal is to reduce the duration of each milking. Several studies have shown that it is possible to reduce milking time by increasing the milk flow switch point (milk flow at which the teatcup is removed) without an impact on somatic cell count and milk yield (3 & 4). One study analysed the effect of increasing the milk flow switch point on milking time in a confinement automatic milking system (5). At this time, the authors are not aware of any research on teatcup removal settings on pasture based automatic milking systems.

The aim of this experiment was to measure the effect of three teatcup removal settings (MFR30, MFR50 and MFR20) on box time, milking time, somatic cell count and milk production rate of cows milked in a pasture based automatic milking system. The MFR30 teatcup removal strategy consisted of removing the teatcup when the quarter flowrate fell below 30% of the quarter rolling average milk flowrate. The MFR50 removal treatment removed teatcups when quarter milk flowrate dropped below 50% of the rolling average milk flowrate and the MFR20 teatcup removal was when quarter milk flowrate dropped below 20% of the rolling average milk flowrate. These settings had a 3 second time delay, thus teatcups were removed 3 second after reaching the milk flow switch point. Three groups of cows (25 cows in each) were assigned one of the treatments for one week, after this time the groups were reassigned to a different treatment. This experiment was conducted during a three-week period, until all groups had transitioned through all treatments. At the end of each week, samples were collected for somatic cell count measurements.

The mixed procedure (Proc Mixed, SAS 9.4 Statements: Reference, Fourth Edition, SAS Institute Inc, NC, USA) was used to test the effect of the treatments on Milking Time, Box Time, Milk Production Rate and Somatic Cell Score.

The MFR30 strategy resulted in a 9 second reduction in milking time and an 11 second reduction in box time compared to the MFR20 removal strategy. The MFR50 strategy resulted in an 8 second reduction in milking time and a

9 second reduction in box time compared to the MFR20 teatcup removal strategy. No differences in milking time or box time were found between the MFR30 and MFR50 teatcup removal strategies. No differences were found between any of the treatments on somatic cell count or milk production rate. This study showed significant reduction in milking time and box time by using a teatcup removal strategy of 30% and 50% of the average flowrate compared to 20% of the average flowrate. This difference in box time for the MFR30 and MFR50 strategies could allow for more than three extra milkings per day. There was no difference in somatic cell score between the early, the MFR30 and the MFR20 teatcup removal strategies. Also, no differences were seen in milk production rate between the three treatments.

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NOTES

DO HERD MASTITIS PATTERNS CHANGE OVER TIME?

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The mastitis “pattern” of a dairy herd can be described in terms of the predominant means of transmission (contagious - C or environmental - E) and whether infections originate from lactation (L) or the dry period (DP). Identifying these patterns is an invaluable first step in improving udder health, allowing targeted intervention. Patterns are identified by analysis of clinical mastitis data and individual cow somatic cell counts (ICSCCs). On a national scale, and on individual farms, patterns may change over time, but to date this has not been formally studied. A new electronic “Pattern Analysis Tool” (PAT) (Breen et al 2017), developed by QMMS Ltd and the University of Nottingham and recently made available by AHDB Dairy (<https://dairy.ahdb.org.uk/resources-library/technical-information/health-welfare/mastitis-pattern-tool>), has enabled rapid herd mastitis pattern analysis. The tool indicates the relative importance of C, EDP and EL patterns, and also the degree of seasonality.

A group of “Sentinel Herds” (Bradley et al, 2017), reflecting the geographical distribution of dairy farms in England, Wales and Scotland, was used to investigate mastitis patterns in the UK in 2012, 2016 and 2017. Recruitment criteria were reliable electronic recording of clinical mastitis and preferably monthly ICSCC recording. Clinical mastitis and ICSCC data from these herds were collated, and TotalVet software (total-vet.co.uk) was used to generate the input parameters needed for the PAT, for each year. Robust data were available to provide 277 mastitis patterns. Only 66 herds could contribute data for all three years, to investigate whether patterns had changed nationally, and on individual farms, between 2012 and 2017, and between 2016 and 2017. Within farms, the patterns demonstrated in the last three months (“current”) and the last 12 months (“recent”) were compared.

The relative distributions nationwide of C v E and DP v L (Table 1) did not vary significantly over the three years (Chi-test $P > 0.1$). The dataset of 277 annual patterns had a similar distribution. However, changes in patterns were seen on individual farms. Only 24/66 farms (36%) showed exactly the same pattern in 2012 and 2017. During this time, 9 herds lost a C component, while 5 acquired a C component. Environmental involvement remained almost constant; only 3 herds acquired, and 3 herds lost, an environmental component. However, the importance of L v DP on individual farms changed over time. Nine herds acquired a DP component, and 12 a lactation component, while 12 lost DP involvement, and 13 lost lactation involvement. Shifts were also seen on 53% of farms between 2016 and 2017, with 3 herds losing and 2 herds acquiring C, 2 losing and 2 gaining E, 13 losing and 15 acquiring DP, and 13 losing and 4 acquiring L involvement. This illustrates

the dynamic nature of infection patterns and the need for constant monitoring.

Table 1 Numbers of herds demonstrating various mastitis patterns for the 12 months ending 31 Dec 2012, 2016 and 2017 (n = 66)

Mastitis Pattern	2012	2016	2017
Contagious (C)	3	2	3
Environmental Dry Period (EDP)	15	8	17
Environmental Lactation (EL)	26	31	30
EL/EDP	15	20	13
C/EDP	1	1	0
C/EL	5	4	2
C/EL/EDP	1	0	1
Some contagious involvement	15.2 %	10.6 %	9.1 %
Some environmental involvement	95.5 %	97.0 %	95.5 %
Some dry period involvement	48.5 %	43.9 %	47.0 %
Some lactation involvement	71.2 %	83.3 %	69.7 %

Period of data analysis was also influential. From 277 comparisons, there were only 129 whose pattern was the same over the past 3 months and the past 12 months. For 18/34 herds with current patterns including C, the annual pattern also included C; for 162/205 herds with current L involvement, the annual pattern also included L; for 72/131 herds with current patterns including DP, the annual also included DP. These findings are not surprising since the tool detected seasonality in the mastitis patterns of 90% of herds.

In summary, epidemiological patterns suggest that the greatest influence on mastitis in the UK is the environment. Although the overall influence of C v E effects, and L v DP does not appear to have altered over a five-year or a one-year period, the relative effects of these influences in individual herds vary considerably both between and within years, emphasising the importance of regular monitoring of udder health.

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NOTES

QUALITY-ASSESSMENT OF *E. COLI* DIAGNOSTICS IN DANISH VETERINARY CLINICS

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SUMMARY

Vaccines can reduce the severity of mastitis caused by among others *E. coli*. However, vaccine success relies on proper diagnostics. This study examines the accuracy of *E. coli*-diagnostics in veterinary clinics in Denmark. The results show a need for increased diagnostic accuracy in mastitis control.

INTRODUCTION

Escherichia coli is one of the most frequently isolated pathogens in clinical bovine mastitis (1, 2). Vaccines such as Startvac improve the defence of cows against mastitis. However, proper diagnostics prior to the vaccine programme is a precondition for vaccine success (3, 4). Accordingly, failure of the vaccine might reflect inaccurate diagnostics. This study examines the accuracy of *E. coli*-diagnostics in veterinary practices in Denmark.

MATERIALS & METHODS

The study examined all milk samples from clinical mastitis diagnosed as *E. coli*, and all milk samples from clinical mastitis which caused diagnostic difficulties in 5 veterinary clinics. The milk samples were collected from late May until 1st of October 2018 and dispatched to the laboratory of Centre for Diagnostics, Technical University of Denmark (CfD) – a part of the Danish Udder Health Center. All milk samples were kept and shipped frozen and routinely processed for microbiological examination at CfD. Contaminated samples (≥ 2 pathogen-types) were omitted from the study. In total, 62 presumed *E. coli* milk samples and 256 milk samples of diagnostic difficulties were included in the study. All pathogens were analysed with matrix-assisted laser desorption/ionization- time of flight (Maldi-tof).

RESULTS

Out of the 62 presumed *E. coli* milk samples 56 were confirmed by Maldi-tof (diagnostic accuracy of veterinary clinic diagnosis = 90 %). Out of 265 milk samples of diagnostic difficulties, 21 were confirmed as *E. coli* by Maldi-tof

(rate of false negative *E. coli* = 8 %). Both false positive and false negative *E. coli* were mainly diagnosis as Gram-positives in the veterinary clinics.

DISCUSSION

This study focuses on the accuracy of *E. coli* diagnoses made in veterinary clinics as they lead to vaccination against *E. coli* and/or false suspicion of lack of vaccine-effect. Considering the importance and omnipresence of *E. coli* the present results are worrisome in two regards: 1) 10 % of the coli-diagnoses are false positives. False positives might lead to improper use of antibiotics and lack of vaccination/misplaced criticism of the vaccine. 2) 8 % of milk samples that cause diagnostic difficulties contain *E. coli*, despite that *E. coli* is not considered easily overlooked. Lack of proper Gram-status in particular points to insufficient diagnostics. These results indicate that diagnostic inaccuracy on *E. coli* might explain lack of vaccine success.

CONCLUSIONS

To improve veterinary diagnostics we need to evaluate the diagnostic quality of major pathogens such as *E. coli*. The Danish Udder Health Centre is currently establishing a large-scale study on the prevalence of mastitis-pathogens in Denmark and the associated diagnostic accuracies.

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NOTES

TEAT DISINFECTION: COMPARISON OF TEAT COVERAGE WITH POST MILKING TEAT DISINFECTANT USING A DIP CUP, VACUUM OPERATED HAND-HELD TEAT SPRAYER AND A PLATFORM MOUNTED AUTOMATIC TEAT DISINFECTANT SYSTEM

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INTRODUCTION

Between June 2013 and March 2018, three evaluation studies were carried out on the efficacy of teat end and teat barrel coverage by three methods of applying post-milking teat disinfectants on UK dairy farms:

a) vacuum operated hand-held spray lance systems; b) automatic platform mounted post milking teat disinfectant system; c) dipping using dip cups.

EVALUATION METHOD

Teat barrel and teat end coverage were assessed post application of the teat disinfectant product using the method described by Pocknee (1).

RESULTS

The study average results for teat coverage end and barrel are given in Table 1, for each of the three studies.

The results for the manual spraying and dipping, confirm anecdotal evidence/observations that dipping is significantly more successful in obtaining significantly better teat barrel coverage – a pre-requisite of obtaining good udder health. The teat dipping results show a very narrow range in efficiency of teat dipping between farms, with an average of 95.3% of all teat barrels being coated in the post milking teat disinfectant. This is in contrast to manual spraying, where there was a range between 19.8 and 83.4% of barrels being covered, with an average of just 50.3%. The platform mounted automatic spray system was significantly better than manual spraying and approaching the success of teat dipping, which provided equal coverage of all four teats and the front and rear planes of each teat. Front teats were often missed with hand held teat spraying.

CONCLUSION

Based on these evaluation studies, teat dipping can rightly be described as the “Gold” Standard against which automatic systems should be compared.

The platform mounted automatic teat spray system provided a much greater degree of consistency in applying teat disinfectant than hand held, vacuum operated teat sprayers. Additional benefits of an automatic teat disinfection system include time saving in the parlour allowing better targeting of labour, with consequential benefits for udder health and milking management. However, the advantages are partly offset by higher chemical consumption.

Table 1. Teat end and teat barrel coverage with disinfectant applied post-milking

	Teat end coverage – score out of 4	Average cover (%) for Left Teats	Average cover (%) for Right Teats	Average cover (%) for Rear Teats	Average cover (%) for Front Teats	Average cover (%) for All Teats
Hand Operated Teat Spraying						
Study Average	3.77	50.06	50.54	52.41	48.19	50.30
Minimum	3.20	18.67	20.96	20.67	18.93	19.80
Maximum	4.00	82.23	85.01	86.19	80.55	83.37
Locate'n'Spray						
Study Average	3.91	81.78	80.90	80.84	81.03	81.34
Minimum	3.84	60.55	63.13	64.48	59.15	61.83
Maximum	4.00	91.05	90.58	90.60	91.03	90.81
Teat Dipping						
Study Average	3.96	95.07	95.29	95.07	95.62	95.28
Minimum	3.92	88.30	90.26	90.63	87.93	89.21
Maximum	3.98	98.45	97.76	98.45	97.86	97.94

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NOTES

MASTITIS IN DEVELOPING AFRICA

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INTRODUCTION

Developing Africa does not have a history of dairy farming. The keeping of local indigenous cattle is important and a sign of wealth. People recognise the nutritional value of milk but it is a luxury. The price of milk varies greatly. In Malawi it's \$0.23/litre while in Ethiopia it can be as high as \$0.70 to \$0.90/litre.

The price of milk needs to be considered with the cost of labour which is in abundance and cheap. the minimum daily wage in Malawi is about \$1 pe day, equivalent of 4 litres of milk a day.

Africa poses a challenging climate with most countries having a rainy season for four to five months of the year and this can be very intermittent. Planted crops often fail as the expected rains do not arrive in time for germination and even then, if the rains do not continue, crops fail. Cows will eat a wide range of forages depending on the type of farmer. Concentrates may also be fed but the quality can be highly variable. Africa is the world's dumping ground for poor quality products and very high prices due to a range of tariffs and corruption in most countries. This paper will focus on small-scale farmers.

SMALL SCALE FARMERS

Farmers can be categorised according to herd size. Small scale farmers will tend to have between one and ten cows and will be hand milking. Hand milking poses a lot of potential problems. It is cheap and easy, no capital outlay, but the spread of Staph aureus infections is high. Cows flick bits of muck and mud into milk. Scouring cows will contaminate even more! Milk is usually strained through gauze to take out much of the physical contamination.

Small scale farmers will have basic facilities and knowledge. Milk is sold locally as either fresh or sour milk, called 'lacto', which is popular. Some of the small-scale farmers will sell milk to larger milk buyers but the quality will be very variable. SCC and TBC are likely to be high. There will be no facility to cool milk and hygiene standards are likely to be poor.

Many of the small-scale farmers keep their cows in a corral by their home. This is to avoid theft or wild animals attacking their stock. For example, in Mozambique and Malawi, the corral will be composed of wooden fences and have clean water and feed area and a simple race type system for milking.

Feed is grown away from the house and is cut every day and brought back to feed the cows. This will require one labour unit. Water needs to be brought back from the well. This might be up to one mile away and with 100 to 125 litres of water needed per cow per day, this will be another labour unit. Milk has to be taken to the milk collection centre which might be as far away as 8 miles. People take this on foot, on a bicycle, donkey or maybe a collection by motorbike if lucky. In some situations, you can use 2 labour units just to take milk to the collection centre.

MILK COLLECTION CENTRES

When the milk arrives at the collection centre it will be weighed and maybe a CMT test carried out. Some centres will carry out alcohol testing as an assessment of bacterial load. This test was first used around 1920. If the milk passes these tests it is poured into the bulk tank at the collection centre which is often powered by solar energy. If the milk fails, it is taken back to the village to sell locally. Often, concentrates will be bought from the collection centre, put into the milk container to feed the cow at the next milking. Milk in, concentrates out!

A key advantage of dairying for small scale farmers is that it offers farmers income every day. This is invaluable in countries where there is no government support or social welfare. Each person must find their own income. This is not an easy life.

PROCESSING AND MILK TESTING

There are many professional dairy processors with quality facilities for milk processing but most struggle with the quality of the raw milk. Electricity supplies and a lack of bulk tank or other cooling facilities means that TBCs can be very high.

Accurate milk quality testing is uncommon. Many will rely on the CMT, alcohol testing for bacteria load and added water. There is some western cell count testing equipment but standard solutions for accurate calibration is rare and maintenance of this equipment will be variable. Test results can be very variable. There are no individual cow cell count testing services unless people buy their own equipment like the DeLaval DCC cell count tester, but even then, the availability and costs of the individual test cassettes are often twice that we pay in the western world. Small holders will rely on CMT testing.

CLIMATE

Climatic variation can be severe. In Mozambique, Zimbabwe or Malawi in the rainy season (November to end of March) you have very high humidity and temperatures of over 35C. Cows suffer significantly from heat stress. Corrals

can become very muddy, you can have up to 150 mm or 6 inches of rain in a day at times, and so environmental clinical mastitis is a problem. In very wet weather cows can literally bury themselves in mud and manure to stay cool and the cow's immune system is under severe challenge.

INFRASTRUCTURE AND SUPPORT

Infrastructure is highly variable and most countries do not enjoy the same facilities that we take for granted in the Western world. Veterinarians are few and far between. Supply of medicines is intermittent including vaccines for controlling important African diseases like Rift Valley Fever, Lumpy Skin Disease and Foot and Mouth Disease. This puts stock at risk. Local cattle wander and act as the vector for many of these diseases.

There are few extension services; but some are provided by local dairy associations, aid organisations or limited government services. Unfortunately, many of these staff are poorly trained and are using materials and information that are out of date. Finances and problems getting visas mean that they cannot get access to the wealth of information that we all take for granted from the likes of meetings like the BMC, NMC, Dairy Expo and other such gatherings to exchange ideas.

MASTITIS PROBLEMS

The biggest mastitis problem is high SCC with *Staph aureus* being the most commonly isolated pathogen, based on limited amounts of bacteriology. *Staph aureus* gangrenous mastitis is commonplace in high SCC herds and causes clinical cases. In the wet season environmental clinical mastitis can be commonplace.

Many are unaware of the Five Point Plan. Quality post milking teat dip can be difficult to obtain and most of very poor quality and often not used. Antibiotic dry cow tubes are expensive and come from dubious sources containing low levels of antibiotic.

High SCC cows can be identified using the CMT test but again, not everyone has access or the ability to carry out this test with some degree of accuracy. Few cows are culled as if you only have three cows, the implication of culling is massive along with the cost of trying to replace the animal with a heifer. All of these limitations mean that the SCC generally is much higher than found on commercial farms.

Despite this, some of the more progressive dairy companies have persuaded and trained small-scale farmers to predip with hypochlorite wiped off with newspaper. Hand milkers will not be wearing gloves but hands are washed and dried between cows to try and limit spread from cow to cow. Cows are then post dipped normally with iodine.

COMMERCIAL FARMERS

Commercial farmers tend to be either white or better educated local Africans. The herd size can be highly variable from 30 cows up to 2,000 cows. They have the same problems with infrastructure.

Most commercial farmers will have milking parlours, tractors and be fully mechanised. They employ large amounts of labour and one worker for every 8 to 15 cows is common. Expect three or four people in any parlour, cow pushers, a team of people feeding, heat spotters, house maids, gardeners, drivers, buyers etc. It's all about creating employment and some of the large-scale farmers will be employing well over 200 people, who are totally dependent on the farmer.

Commercial farmers will house their cows in corrals and a few have freestall barns, but these are uncommon. Shade for cows is variable. Trees are ideal to offer some shade from the sun in the middle of the day, but many farmers cut these down to make more space. Shade over the feed areas is becoming more popular to preserve the freshness of food and maximise feed intakes.

Electricity is always a problem as is a plentiful supply of clean water. Farmers need their own generators which are expensive to run. They will often irrigate crops. The progressive farmers can afford to get outside advice and visit other countries to learn and some do, others do not.

Many commercial farmers will use mixer wagons but most of these are quite elderly and in need of a lot of TLC. The real problem with feeding cows is the poor quality of the raw ingredients as these are dumped on Africa. Nutritional content can be very variable. Key ingredients run out of stock and so at times rations must change overnight. The rumen of the cow suffers. Ration formulation can be very challenging.

Commercial farmers are likely to have a range of enterprises; potatoes, crops, tobacco, fruit, beef and this helps when there are market variations. Staff tend to be better trained and they will have housing provided along with a small amount of land to grow maize for their family. Some larger farms employ their own teachers, doctor etc. Commercial farmers generally look after their staff well, but if staff steal or break the rules they are sacked on the spot and must leave with their family.

NOTES

MEETING EXPECTATIONS WITH THE TEAT DISINFECTANTS OF TODAY

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SUMMARY

- Modern teat disinfectants are complex formulations that have multipurpose
- A good teat disinfectant has to kill bacteria quickly (germicide activity), spread over the skin easily (surfactant), and ensure teat skin is healthy, soft and undamaged (emollient)
- Modern disinfectants have a formulation that can be sprayed as well as dipped, if the correct spray system is used, to ensure good skin coverage as soon as possible after milking
- Manufacturers are responsible for developing and commercialising pre- and post-milking products that meet producer needs, but that are also well aligned with market and regulatory demands.

INTRODUCTION

We are constantly reminded of the fast-paced and evolving environment that we live in. Some dairy farmers are milking bigger cows that yield more milk than ever before, others are milking more cows, and we are all trying to harvest the milk in the fastest way possible, using either manual or automated means. Efficient use of labour is becoming vital, often because skilled help is harder to find. All that, and we also expect our cows to remain healthy for the largest number of lactations possible. Moreover, if these cows get sick, we want to make sure we reduce (or nullify if possible) the usage of antibiotics – as consumers consistently demand of livestock products in a growing number of countries. In terms of mastitis prevention, usage of an effective teat disinfectant product, applied in the most appropriate way and at the correct time, is essential. This paper summarises the advances in teat disinfection and the extra value in modern formulations.

In a series of experiments conducted by Frank Neave and the mastitis team at NIRD in 1962, it was clearly shown that the amount of teat end contamination by bacteria is directly related to the risk of an intra-mammary infection (IMI), effectively a direct correlation between the numbers of mastitis-causing bacteria and expected IMI. Getting teats clean and reducing the numbers of bacteria involved in mastitis that occur on and in teat skin has remained an essential part of achieving a healthy udder and good quality milk ever since.

Teat disinfection has and will remain one of the key components of mastitis control and ensuring production of safe milk. The main components of a teat disinfectant have been explained previously (Lopez-Benavides, 2014). In essence, a teat disinfectant is composed mainly of three things: a) a germicidal component for killing microorganisms, b) an emollient package for maintaining good teat skin health, and c) a surfactant element for better coverage on teat skin and for improved removal of soiling material such as mud or faeces. Water makes up the majority of the formula and the importance of the chemical and physical properties of that water, especially its quality when the disinfectant is prepared on farm from a concentrate, must not be underappreciated. Other functional elements of teat disinfectants help to position a product for a specific role within the milking routine. These will be discussed in more detail in this paper.

Germicidal components

A disinfecting solution applied to teats when the cow had finished milking was the first really successful attempt at preventing mastitis in cows. Pine oil was first used in 1916, and years later a dilute solution of sodium hypochlorite (bleach) became the germicide of choice. Simple iodine formulations were introduced in the 1960s. Many different germicides have been suggested and tested over time, but only a few have become solid commercial candidates due to market acceptability and regulatory demands that focus more and more on efficacy and preventing contamination of milk and its products. Examples of germicides having some to a lot of use include: hydrogen peroxide (Leslie *et al.*, 2006), but not allowed in some countries as it has been used to 'clean' bulk milk because it is largely undetectable (peroxides degrade to water and oxygen); DDBSA (dodecylbenzenesulfonic acid), not so common now as a post-milking product as it is akin to a swimming pool disinfectant and can result in residues, although it has proven to be an excellent pre-milking teat disinfectant with desirable cleaning and germicidal properties (Galton *et al.*, 1986a; Bruno and Lopez-Benavides, 2015; Lopez-Benavides *et al.*, 2015); chlorhexidine, an older technology still very common in many countries (Hogan *et al.*, 1995), but being challenged due to its use in human medical hygiene; and more modern disinfectants that use chlorine dioxide (Oliver *et al.*, 1993) or lactic acid (McPhee *et al.*, 2015; Watters *et al.*, 2015) or glycolic acid (Lago *et al.*, 2016; Godden *et al.*, 2016). Iodine is still the preferred germicide in most countries. The early formulations, being extremely acidic, were harsh skin irritants. The modern formulations are closer to a neutral pH and so milder on skin, but they also have enhanced killing power as they liberate more free iodine at a lower overall iodine content.

A summary of the most common oxidative-type germicides is shown in Table 1.

Table 1. Mode of action of oxidative type germicides

Germicide	Proposed mode of action which lead to bacterial death
Iodine	<ul style="list-style-type: none"> • Oxidation of sulphur-hydrogen groups in some amino acids which leads to inhibition of protein synthesis • Iodination of phenolic and imidazolic groups in some DNA which leads to DNA denaturation (separation from double to single DNA strands). • Precipitation of the proteins of the microorganisms by forming salts via direct halogenation. • Interaction with phospholipids causing damage to the cell wall and loss of intracellular material.
Chlorine dioxide (and chlorine compounds)	<ul style="list-style-type: none"> • Formation of chloramines, when chlorine compounds mix with bacterial protoplasm. • Halogenation or oxidation reactions with bacterial cells, causing change in cellular permeability and affecting vital enzymatic systems. • Cessation of protein synthesis in growing bacterial cells.
Hydrogen peroxide	<ul style="list-style-type: none"> • Chemical oxidation of cellular components. Hydroxyl radicals produced in the reaction attack cell membrane lipids, DNA and other essential cell components. • Oxidation of sulfhydryl groups and double bonds in proteins, lipids, and surface membranes.

The emollient package

A fundamental outcome of the early Neave work was that ensuring intact and healthy teat skin, e.g. preventing chapping of teat skin, was critical in minimising mastitis-causing bacteria, especially *Staphylococcus aureus* and *Streptococcus dysgalactiae*, on teat skin. In experimental studies, intentional chapping of teats with sodium hydroxide caused aggressive dryness to skin, and teats that were more likely to support colonisation of *Staph. aureus* (Fox and Norell, 1992). Faster healing and lower bacterial colonisation of these teats was observed when they were dipped in solutions with emollients.

The emollient package is associated with maintaining good teat health and preventing skin dryness, but overall it is the formula that has a beneficial or adverse effect on teat skin health. A 5% glycerine teat disinfectant was used on two automatic milking system (AMS) farms that had not used any product for more than a year. The result was that, in as little as two weeks, optimal condition of both the teat barrel skin and the teat end), was achieved, becoming more than 2.2 times greater than initial values (Geldhof *et al.*, 2018).

It is the responsibility of manufacturers to commercialise products that have the expected properties needed for pre- or post-milking application. In practice, emollients make more sense when applied post-milking than before milking because of longer contact time, a consideration that producers need

to think about. Typically, emollient levels in post-milking teat disinfectants do not exceed 10%, unless they are a formulation available in some countries to cope with extreme winter conditions, known as winter dips. Because effective teat disinfection is a result of the formulation and its many chemical interactions, it is not guaranteed that the inclusion of higher levels of emollients alone will guarantee better skin conditioning. In some cases, emollients are used to mitigate the harsh effects that some other teat dip components may have on skin (see NPE in next section). Teat condition evaluation is important to guarantee the safety of products on teat skin and we recommend that this be done regularly to know the teat skin condition of the herd, establish goals, and take action to meet them. In modern, well-managed herds that have milking machines in proper working order and use well tested products, a teat condition score of 1 (optimal teat skin) should be evident in 90-95% of quarters.

Surfactant elements

Surfactants are molecules that may play various roles within a teat disinfectant formulation, such as a detergent (helps to remove soil from the skin), a solubilising agent (complexing agent in iodophores), a foaming agent, or an emulsifying agent (Mishra *et al.*, 2009). For cleaning purposes, for example, by helping to reduce the superficial tension between the liquid solution and the skin, better penetration of the product into the immediate skin surface is achieved, leading to better removal of anything soiling the skin. Teat disinfectants with cleaning claims (especially for the pre-milking routine) should have a surface tension lower than water, and this should be evident in the success of cleaning compared with water alone. Gentinini *et al.* (2018) showed the benefit of using a properly formulated cleaner in the teat cleaner cup of the DeLaval VMS. The surfactant-based product was approximately five times more likely to clean dirty teats (scores 3 and 4 in scale of 1-4 by Hovinen *et al.* 2005) than water alone.

The levels of inclusion of surfactants in a formula vary according to the expected function of the product. Another interesting property of surfactants is their wetting ability. By lowering the surface tension of the liquid, the product achieves better coverage on skin when dipped or sprayed onto teats. This is the same action seen in plant sprays to get whole leaf coverage from droplets and hence why these disinfectants can be sprayed onto teats successfully.

Probably the surfactants that have received most attention in the dairy world in the last 2-5 years are the nonyl-phenyl ethoxylates (NPE), commonly used in industrial laundry detergents and furniture plastics, but also used for decades (and still by many manufacturers) as complexing agents for iodine. The NMC (2016) has issued a factsheet on NPE. Iodine products with NPE tend to be harsher on skin and teat condition can be affected. In many cases, manufacturers may try to mask this negative effect by increasing emollient levels in the formula. A recent teat condition study (Sima and Lopez-Benavides, unpublished) over a three-month period showed that teats dipped

with an NPE-free iodine product pre and post milking were 3.4 times more likely (95% CI 2.6-4.5) to have better teat skin condition than when an NPE iodine product was used in the same farm environment. Because of the current market environment where NPE products are not wanted, it is very likely that we will see fewer and fewer commercial products containing NPE.

Pre-milking teat disinfection

Pre-milking teat preparation is arguably one of the most important activities in the whole milking routine. Handling of teats stimulates release of oxytocin to ensure milk let-down, fore-stripping helps to identify abnormal milk and application of a pre-milking product aids in the reduction of bacteria on teats (Galton *et al.*, 1986). Depending on the product used and germicide present in the pre-milking disinfectant, use of a pre-dip was 1.2 to 4.5 times more likely to reduce *Staph. aureus* and *Streptococcus* spp. counts compared with washing and drying alone (Gleeson *et al.*, 2009). Results from pre-milking disinfection are more variable between farms (Pankey *et al.* 1987, Hillerton *et al.* 1993) but when any farm has a mastitis problem all use of a good disinfectant product is good.

Applying a teat disinfectant

Mostly we talk about teat dipping and this remains the most common means of applying a disinfectant in small herds, and almost exclusively in some countries, e.g. USA. However, when the parlour has a larger throughput or is labour-limited, e.g. large rotary parlours with only one person at cups-on position, automated means of disinfecting teats are needed, added to cluster removal, etc. Traditionally, spraying disinfectant was hit and/or miss, commonly in an exit race, and consumption with such systems usually used two or three times the amount of disinfectant applied by dipping (4-10 mL versus up to 30 mL per cow per milking)! Fortunately, efficient automated disinfection systems, very soon after cups-off and whilst the cow is still on the platform, are now available. One system, in use on conventional and automated rotary milking farms, uses a single robotic arm. Another, now found internationally, uses individual spray nozzles located below each teat whilst the cow is still in the milking position. These systems solve one of the requirements of the earliest work on teat disinfection: do it as soon as the cluster comes off. They may also use a similar amount of disinfectant to teat dipping.

The mode of use and application of pre/post-milking teat products is distinguishable between AMS manufacturers. Teat cleaning may involve air, vacuum and a cleaning solution while pre/post-milking disinfection is done separately by spraying (DeLaval VMS). Another manufacturer relies on a cleaning/disinfecting solution delivered via a rolling brush mechanism to prep teats, and sprays teats after milking (Lely Astronaut 5), while yet another focuses on doing all the necessary functions in an all-in-one, inline-type approach (GEA Monobox). Manufacturers consider the needs of the consumer and the market to deliver solutions for pre- and post-milking teat application.

In the end, a teat disinfectant should have enough contact time to kill the microorganisms on the teat skin (having a broad spectrum activity against the wide array of mastitis pathogens), the surfactant has to help remove soiling from dirty teats and facilitate a clean and dry milk harvest, and the emollient has to cover as much of the teat as possible to maintain a healthy and soft skin.

Current and future demands

Accuracy in teat disinfectant application when dipping has always been considered better than spraying, if done properly. But modern spray systems outcompete poor dipping and challenge good dipping. When opting for automatic and/or spray systems, the factors to be considered when choosing one system over another include, in order of importance: 1) accuracy of delivery (identifying a teat and providing adequate coverage, especially to the teat end), 2) risk of residues in milk (dependent on types of chemicals used together with amount delivered), and 3) consumption (Lopez-Benavides and Paulrud, 2018). It is evident that machine learning will ensure an improvement on accuracy at more demanding time constraints in automated systems. Unwanted chemicals in milk (by consumers and/or regulatory agencies) should be seriously considered by the producer (summary in Table 2). Such is the case for NPE, surfactants commonly used in teat dips and detergents (banned by many milk buyers in countries from China to the USA). Other chemicals not naturally present in milk and used in dairy operations will likely also be questioned. Development of efficacious and consumer-safe teat disinfectants are the responsibility of manufacturers working jointly with regulatory authorities in different countries.

Table 2. Germicides commonly present in teat disinfectants and risk residues (Source: Hemling (2015))

Germicide	Natural in milk	% in teat disinfectant (ready to use)	Other	Germicide as residue in milk
Iodine	Yes	0.1-1%	NPE	No – converts to iodide
Hydrogen peroxide	Yes	0-10%		No – decomposes
Chlorine dioxide	No	0.5 chlorite: 100-200 ppm chlorine dioxide	Chlorite residue	No - decomposes
Chlorine	No	0.05-2%	THM - chloroform	No - decomposes
Lactic – organic acid	Yes	2-6%		Not germicidal at milk pH
Chlorhexidine	No	0.3-0.5%		Yes
DDBSA	No	2-4%		

The presence of iodine in milk above recommended values (500 ppb, World Health Organization) is an issue that has been raised repeatedly over many

years, and even more so recently because of concerns for infants that consume milk prepared from powder. An obvious link between higher levels of iodine in milk and the usage of these products on farm has been suggested (Borucki Castro *et al.*, 2010). Earlier studies showed that when pre-milking udder preparation is not done, quarter iodine levels were 143 and 291 ppb when quarters were dipped with a 1,000 or 5,000 ppm iodine product applied post-milking, respectively. With a complete routine (cleaning and wiping), iodine levels were 70 and 99 ppb, roughly 50% lower (Sheldrake *et al.*, 1980). Later studies evaluated the effect of using 1,000 ppm (0.1%) or 10,000 ppm (1%) iodine products pre- and post- milking. While the use of 0.1% pre-milking product did not significantly increase iodine levels compared with 0.1% iodine used post-milking, the use of a 1% iodine product pre-milking resulted in a significant increase in iodine levels in milk (Galton *et al.*, 1986b). More recently, French *et al.* (2016) compared iodine residues in milk when iodine products that differed in concentration and method of application were used. Their conclusions supported that the majority of iodine in milk most likely originates from the feed. Iodine residues in milk when no products were used ranged between 145-182 ppb. More iodine in the teat disinfectant is positively correlated with higher iodine residues in milk, but the overall increase reported was only 8-29 ppb (French *et al.*, 2016). A similar increase (~20 ppb) over a base average of 200 ppb was observed in a study when a 1,500 ppm iodine pre-milking product was used (Lopez-Benavides *et al.*, 2016). Lessons learned from these studies have practical implications: a) dipping teats will lead to lower residues compared with old style spraying when three time the disinfectant volume is used, b) use of iodine products pre-milking increases iodine levels in milk, more so when teats are not wiped after disinfection, c) higher iodine content products will result in higher levels of iodine in milk. These implications should be considered when advising dairy farmers on the management of iodine (or any other germicide) in their bulk tank. Strangely this is no different from work some 30 years ago at Compton by Bob Grindall (unpublished) and early work by Kieran Jordan of Teagasc, recently repeated (O'Brien *et al.*, 2013), showing the above and that diet can lead to a greater range of iodine in milk than teat disinfection.

Protection of teats by creating a film on the teats lasting between milkings by including a barrier component in the teat disinfectant is used by many dairy farmers in some markets, especially when outbreaks of environmental mastitis is a risk. A surge of studies on barrier-type products occurred in the 1990s and many products became available commercially worldwide. Nowadays, barrier-type products are used by 46% of large dairy operations in the USA. Around 52% of these operations use barrier-type products all year round, the rest only use them in either adverse weather or on a select group of animals (USDA, 2016). Properties associated with barrier products include viscosity, seen as gloopiness (assumes that more viscous products are better barriers), colour intensity and persistence. Barrier products should show evidence of a physical film that adheres to teat skin uniformly and is backed up by field data on efficacy. An added benefit is that the germicidal component is still active in the film, even when dry. Colour intensity may be a useful tool to monitor milking routines, e.g. identify cows that have been

dipped/not dipped properly by milkers and/or not teat-prepared properly because the teat is still coloured.

CONCLUSION

- Modern day teat disinfectants do more than kill bacteria on teats, still an essential function of any product used pre and post-milking.
- Healthy and soft teat skin should be a target of any dairy, and this is achieved by using products that are not harsh to skin and have an acceptable level of emollients.
- Surfactants play an important role in teat disinfectants, from complexing iodine, foaming properties, and by improving teat coverage.
- The overall benefits of any well formulated product can only be achieved if applied properly on teats, whether by manual or automated means.

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NOTES

AHDB MASTITIS CASE STUDY: REDUCING CLINICAL MASTITIS RATE AND ANTIBIOTIC USE IN A SOMERSET DAIRY HERD

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SUMMARY

Clinical mastitis rate and antibiotic use were reduced following implementation of a plan based on the AHDB Dairy Mastitis Control Plan. In July 2017 the clinical mastitis rate was running at 83 cases/100 cows/year and the farm antibiotic use had been highlighted during routine benchmarking carried out by the veterinary practice. Data analysis led to a diagnosis of environmental mastitis of predominantly dry period origin and a farm visit was carried out to identify the major risk factors. A list of control measures were prioritised following discussion with the farmer and his routine veterinary surgeon. A year later, the clinical mastitis rate has dropped to 39 cases/100 cows/year, lactating cow tube use has dropped from 3.90 to 0.66 DCDVet and the farm is no longer in the top 10% of antibiotic users in the practice. Subsequent reviews have identified lactation origin mastitis as currently the biggest problem and control measures have been implemented to reflect this.

INTRODUCTION

The AHDB Dairy Mastitis Control Plan (AHDB DMCP) is considered to be the gold standard in mastitis control (2), but its implementation on farm has been limited due to poor up-take by vets and farmers. The reasons for this have been the subject of some conjecture but could be linked to a perceived unwieldy structure and a high up-front cost to the farmer, despite a demonstrable cost benefit (1). Recent research has suggested that the approach has been used far more widely than has been captured by the support team in terms of plans completed (4). As a result a lighter approach has been suggested which nevertheless follows the same principles of data analysis, diagnosis, farm visit, prioritised control measures and continuous review. This case study describes such a mastitis control plan, implemented following the principles of the AHDB DMCP, which has resulted in a significant reduction in clinical mastitis and also in antibiotic use as measured by mg/kg PCU, and in particular DCDVet/cow/year.

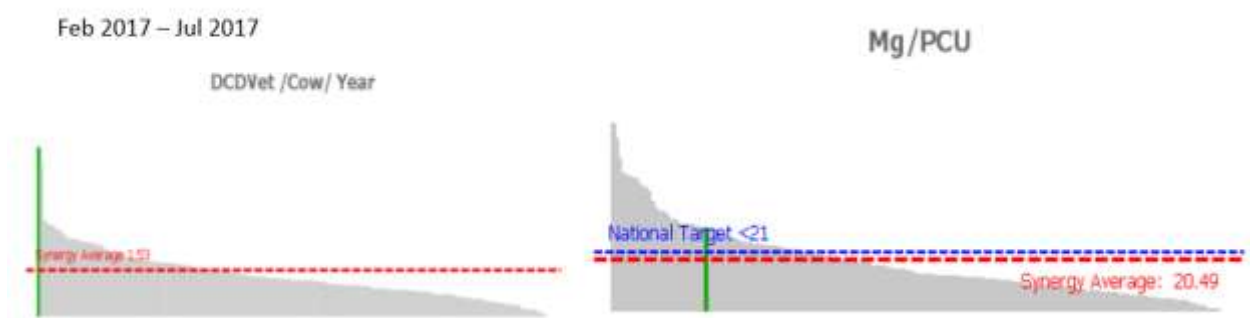
Furthermore there has been pressure on the industry to reduce antibiotic use as part of the attempt to halt the spread of antimicrobial resistant organisms (5). Analysis of antibiotic use by client has been carried out at Synergy Farm Health (SFH) for several years to aid in health planning and as a benchmarking exercise (3). In 2017 RUMA produced targets for the dairy

industry to achieve by 2020, which included reducing total antibiotic use below 21mg/PCU and lactating intramammary tubes below 0.727 DCDVet (6). Although these are industry aims and average antibiotic use at SFH was shown to be well within these targets, there were a number of individual farms still not meeting them. Therefore, attention has been focussed on antibiotic reduction in general, and on those farms exceeding these targets in particular.

MATERIALS & METHODS

The farm had been highlighted during routine antibiotic benchmarking as being one of the highest antibiotic users in the practice. The antibiotic use relating to total antibiotics used in mg/kg PCU and DCDvet are shown in Figure 1 and the number of DCDVet of lactating cow intramammary tubes per cow per year was estimated at 3.90, compared with the RUMA target of 0.727 and the SFH mean of 0.65. It was suggested to carry out an investigation as part of a student teaching exercise, following which data analysis and a risk assessment were carried out. Control measures were then prioritised following a meeting with the farmer and the routine vet. One year on there are regular quarterly reviews to assess progress and re-evaluate the most effective control measures.

Figure 1: Antibiotic use shown as a green bar, compared to other SFH clients as expressed in DCDVet/cow/year and mg/PCU, for the 6 months to July 2017



Data Analysis

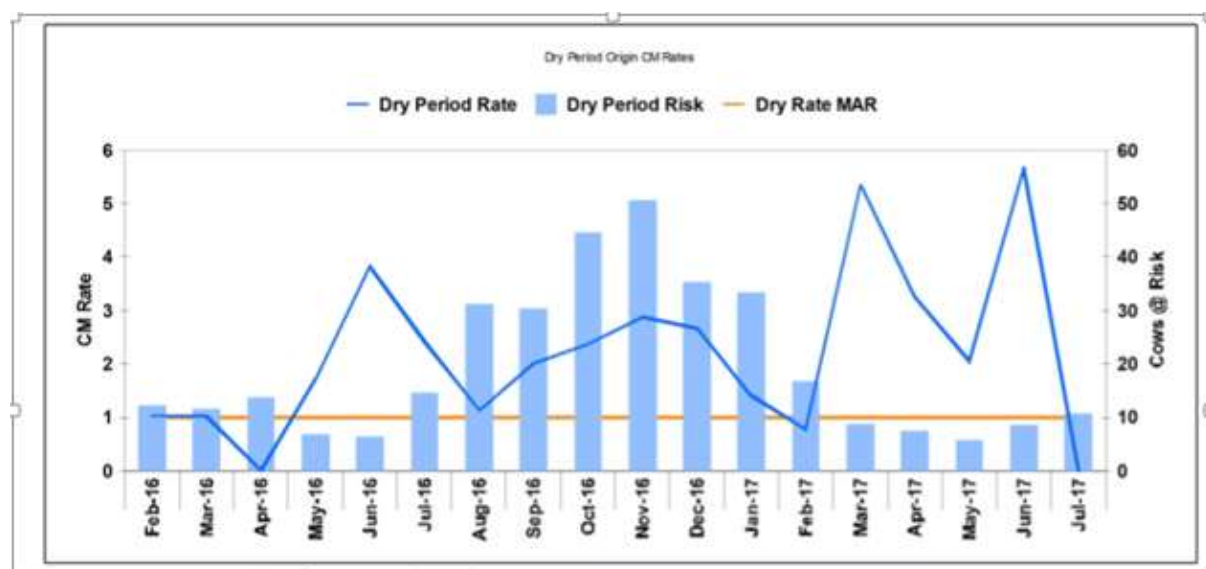
The mastitis key performance indicators (KPIs) at the farm in July 2017 are shown in Table 1.

Table 1 Mastitis KPIs in July 2017

Parameter	3 month rolling average	Annual rolling average	Target
Clinical cases/ 100 cows/year	89	83	<30
Dry Period Origin Rate/12 cows	3.96	2.33	<1
Lactation Period Origin Rate/12 cows	2.03	3.08	<2
Dry Period New Infection Rate	25.0	15.6	<10
Lactation New Infection Rate	6.6	9.5	<5
Dry Period Cure Rate	72.7	62.1	>85
% Herd Chronically Infected	15.4	16.2	<5
% Herd >200,000 cells/ml	19.0	23.2	<20

The index rates of clinical mastitis of dry period and lactation origin are shown in Figures 2 and 3 respectively. It can be seen that while both were over target, mastitis of dry period origin was over target more consistently and by a greater margin. It was therefore decided to tackle this area first.

Figure 2: Dry period origin rate of clinical mastitis for the 18 months to July 2017.



The blue bar represents the number of cows at risk (less than 30 days calved), the blue line represents the dry period origin incidence and the orange line represents the maximum advisory rate of 1 case for every 12 cows calving.

Figure 3: Lactation origin rate of clinical mastitis for the 18 month period to July 2017.



The green bars represent the number of cows at risk, the green line represents the lactation origin incidence and the orange line represents the maximum advisory rate of 2 cases for every 12 cows eligible.

On Farm Risk Assessment

A farm visit was conducted in July 2017 and again 2 weeks later as part of a teaching exercise. The lactating and dry cow environments were assessed, milking routine observed and approximately 20 high cell count cows sampled for bacteriology. Observations were made and questions asked of the farmer regarding straw yard and cubicle bedding management, stocking rates, pasture management, cow cleanliness, management around calving, pre- and post-milking routines and basic parlour function.

Bacteriology results are shown in Table 2

Table 2 Bacteriology results from high cell count cows

Result	Number of isolates
Staph aureus	2
Strep uberis	4
Bacillus sp	3
No Growth	9

Prioritisation of Control Measures

A list of control measures was drawn up and a diagnosis of environmental mastitis of predominantly dry period origin was made. This list was then shortened to focus on the priorities that were likely to make the biggest impact in reducing mastitis. It was really important to target not those measures that would be easiest to implement, but those that were thought to be the most important. Eight priorities were agreed, as listed below:

- Move dry cows at grass to a different field or move feeders to avoid severe poaching.
- Calving yard stocking rate: there should be 15 sq. m. per cow.
- Straw yards should be cleaned out completely every 3 to 4 weeks.
- Cows should be milked within 12 hours of calving if possible.
- Ventilation in the calving yard and milking cow shed should be improved.
- Fresh-calved cows should be moved to comfortable cubicles NOT a straw yard reserved for lame, fragile and fresh-calved cows.
- The agreed milking routine needs to be implemented consistently by all milkers as follows: foremilk, pre-spray, wipe, apply cups. Split the line in two to keep lag times to around a minute.
- Review in 3 months' time.

RESULTS

Implementation

- During the summer, dry cows were housed in cubicles at the start of the transition period, to avoid the need for ring feeders in the field.
- Straw yards were cleaned out at least monthly and stocking rates reduced by adding cows later during busy times.
- Cows were milked on average one milking sooner after calving, aiming for less than 12 hours after calving.
- Fans were installed in the milking cow cubicle shed; no changes made to the calving yard ventilation.
- New cubicles with water-filled mattresses and sawdust bedding were installed for the fresh cow group. The straw yard was reallocated to heifer accommodation.
- Staff changes resulted in a new herdsman in October 2017. Recommended changes to the milking routine were implemented at the same time.
- Quarterly reviews were carried out.

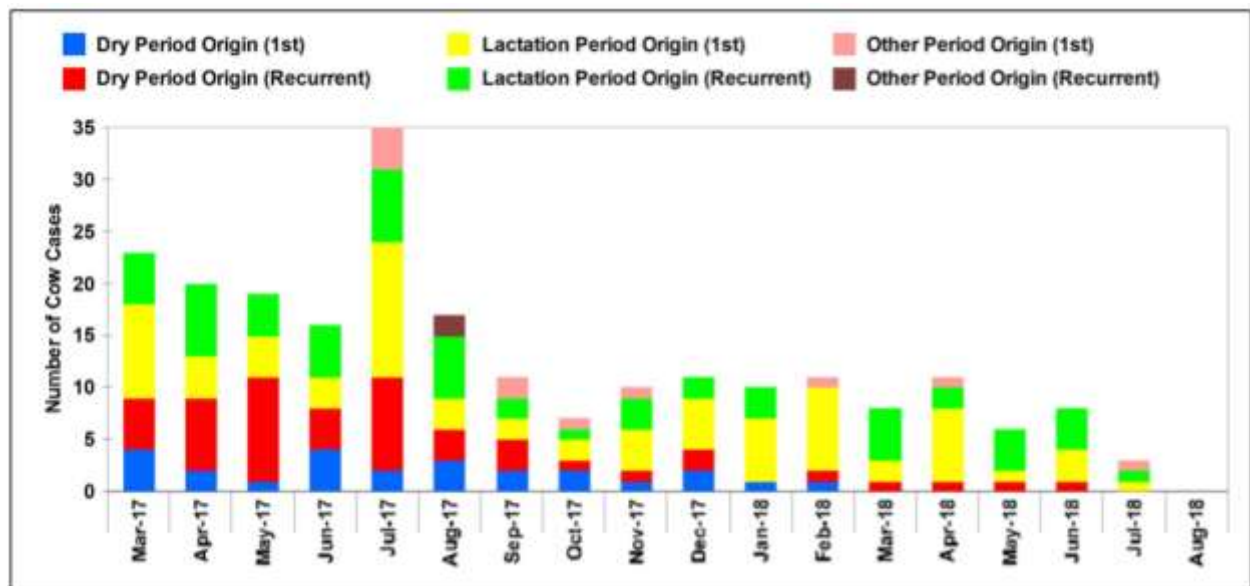
Clinical mastitis and cell counts

Clinical mastitis and cell count figures as at August 2018 are shown in Table 3. The mastitis rate fell from 83 cases/100 cows/year to 39 cases/100 cows/year in a 12 month period and it can be seen that much of this reduction came from DPO mastitis. Clinical mastitis incidence by month can be seen in Figure 4 and the index rates of clinical mastitis of dry period and lactation origin are shown in Figures 5 and 6 respectively. The dry period sub-clinical new infection rate is shown in Figure 7.

Table 3 Mastitis KPIs in July 2018

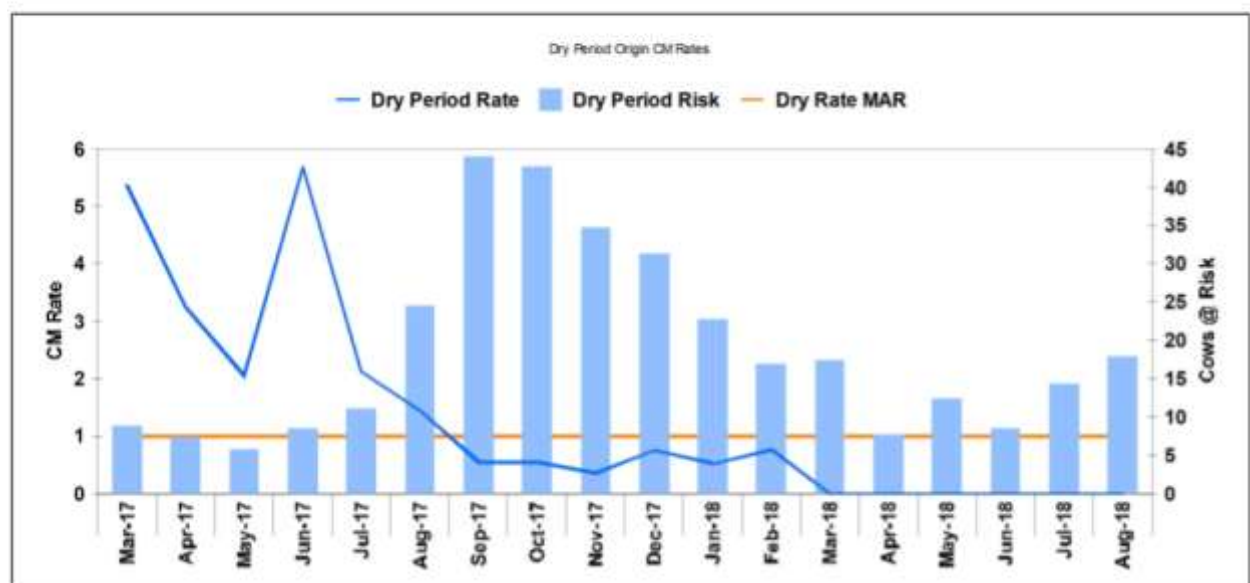
Parameter	3 month rolling average	Annual rolling average	Target
Clinical cases/ 100 cows/year	24	39	<30
Dry Period Origin Rate/12 cows	0.00	0.52	<1
Lactation Period Origin Rate/12 cows	0.88	2.15	<2
Dry Period New Infection Rate	13.6	14.6	<10
Lactation New Infection Rate	9.9	8.2	<5
Dry Period Cure Rate	94.4	74.6	>85
% Herd Chronically Infected	13.4	13.5	<5
% Herd >200,000 cells/ml	24.8	20.7	<20

Figure 4: Clinical mastitis incidence by month in the 18 months to July 2018.



The blue bars represent index cases of dry period origin and the red bars recurrence therefrom; the yellow bars represent index cases of lactation origin and the green bars recurrence therefrom; the pink bars represent index cases occurring after 300 days in milk.

Figure 5: Dry period origin rate of clinical mastitis for the 18 months to August 2018.



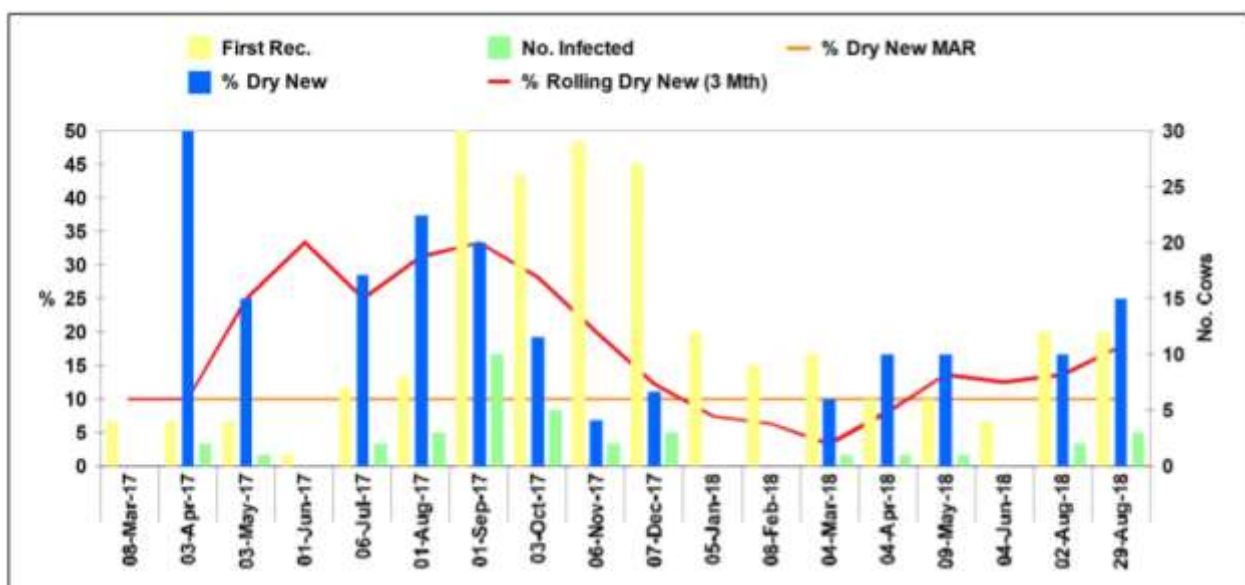
The blue bar represents the number of cows at risk (less than 30 days calved), the blue line represents the dry period origin incidence and the orange line represents the maximum advisory rate of 1 case for every 12 cows calving.

Figure 6: Lactation origin rate of clinical mastitis for the 18 month period to August 2018.



The green bars represent the number of cows at risk, the green line represents the lactation origin incidence and the orange line represents the maximum advisory rate of 2 cases for every 12 cows eligible.

Figure 7: Dry period sub-clinical new infection rate in the 18 months to August 2018.

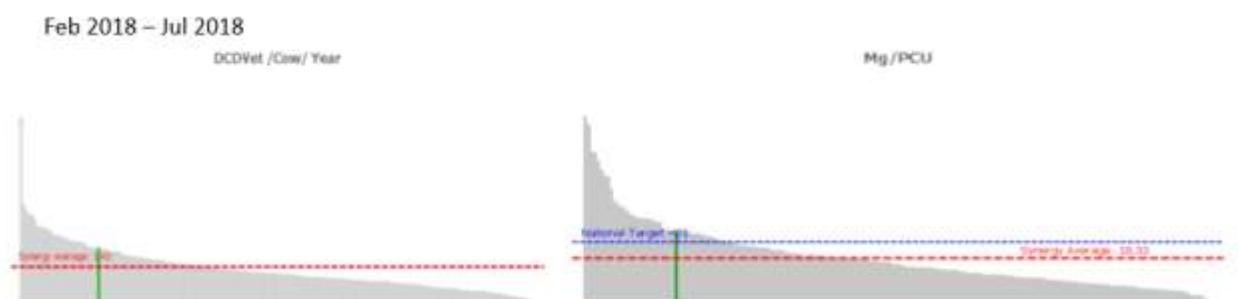


The yellow bars represent the number of cows at risk (less than 30 days in milk), the green bars represent the number of new infections and the blue bars express this as a percentage of the cows at risk. The red line represents the 3 month rolling average and the orange line is the maximum advisory rate of 10%.

Antibiotic use

Lactating cow intramammary tube use as measured by DCDVet/cow/year reduced from 3.90 to 1.60 in the 12 months to July 218. This figure has continued to fall however, as the 6 monthly figure to July 2018 is 0.66 DCDVet. The antibiotic use relating to total antibiotics used in mg/kg PCU and DCDvet is shown in Figure 8. As would be expected when reducing mastitis treatments, there has been a bigger impact on antibiotic use when measured in DCDVet than in mg/PCU. This is because a course of lactating cow intramammary tubes applied in a mastitis case contains less total antibiotic than a course of parenteral antibiotics.

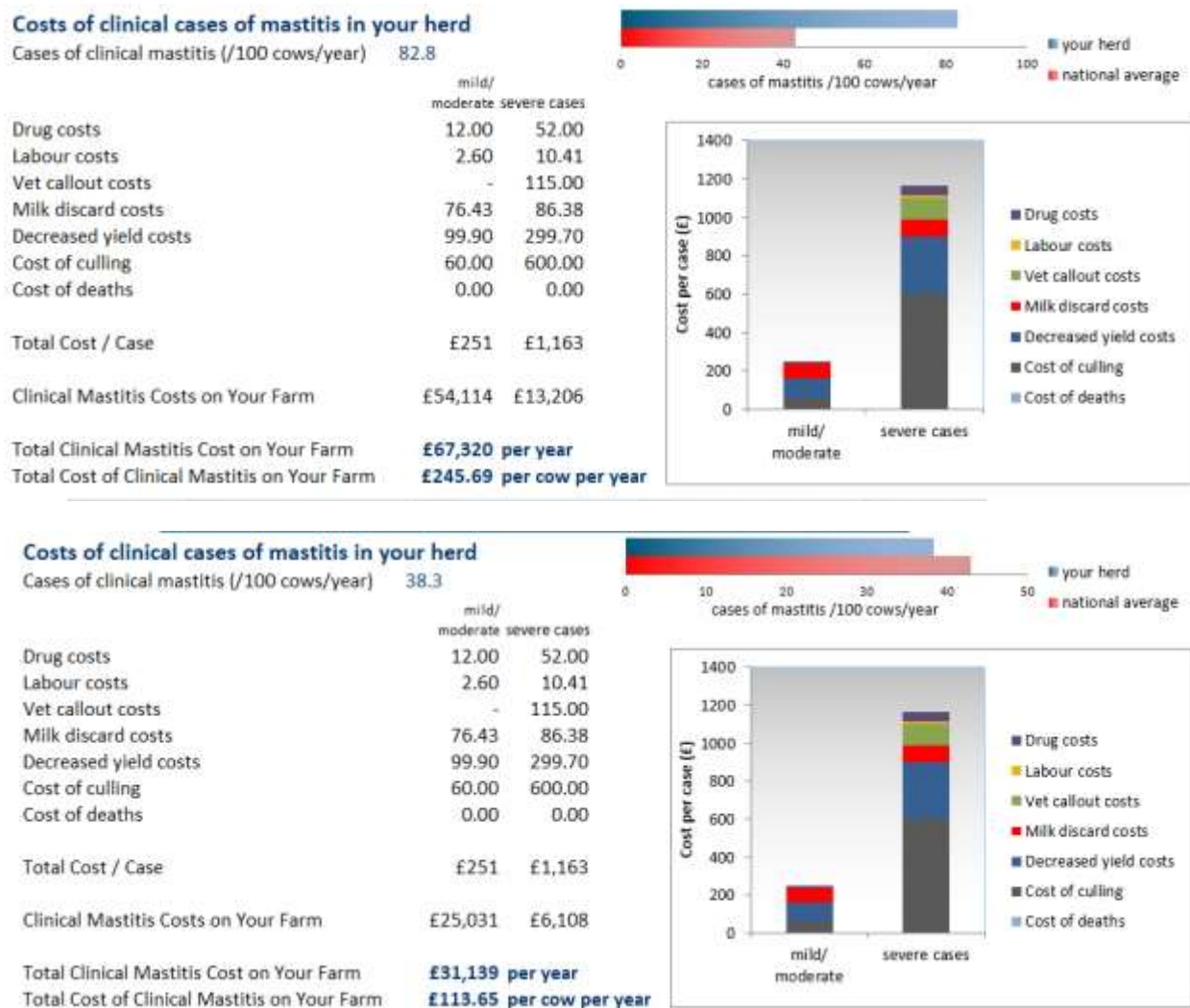
Figure 8: Antibiotic use shown as a green bar, compared with other SFH clients as expressed in DCDVet/cow/year and mg/PCU for the 6 months to July 2018



Economic Assessment

Farm specific figures were used to estimate the cost of clinical mastitis on the farm in the period both before and after the original intervention. These are shown in Figure 9 and demonstrate a reduction in costs associated with clinical mastitis of £36k between July 2017 and July 2018.

Figure 9: Comparison of the cost of clinical mastitis in the 12 months to July 2017 (above) and July 2018 (below) using the AHDB DMCP cost benefit calculator tool



DISCUSSION

It can be seen that a series of control measures aimed primarily at bringing down the rate of clinical mastitis of dry period origin have been extremely effective in achieving this goal, resulting in a reduction in antibiotic use and significant economic savings.

Subsequent reviews have also led to the phasing out of Highest Priority Critically Important Antibiotics (HP-CIAs), the instigation of selective dry cow therapy and the introduction of an on-farm culture system for aiding treatment decisions. More recent reviews have identified clinical and sub-clinical mastitis of lactation origin to be the major issues and control measures have been formulated to address this. These include measures to improve cow cleanliness, such as increased frequency and quantity of bedding up, changes to the sawdust quality, and a review of mastitis detection and

treatment protocols. In other words mastitis control has become a process of on-going assessment and review.

CONCLUSIONS

This case study demonstrates how antibiotic sales can be replaced by advisory work on mastitis control, to the benefit of both farmers and vets. A simplified approach based on the AHDB DMCP can be very effective in bringing down clinical mastitis rates and antibiotic use, although the original plan remains the gold standard for mastitis control in the UK.

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NOTES

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INTERNET OF THINGS ENABLED EARLY DETECTION OF MASTITIS

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SUMMARY

Wireless technologies have enabled the creation low cost sensors and decision support tools that enhance farm operational efficiency. Sensor networks are becoming internet enabled facilitating data sharing. Hence, a range of novel and/or enhanced services are being made possible. Validation trials on a commercial farm suggest that early detection of mastitis is possible 1-2 days in advance of a human operator.

INTRODUCTION

Automated (oestrus) detection systems are now commonplace on dairy farms [1-3]. Many vendors have further processed motion data to provide information on activity, feeding and rumination times. This information can be offloaded through a range of mobile interfaces. A significant opportunity exists to enhance the performance through integrating disparate sensor data streams to optimize performance.

MATERIALS AND METHODS

Activity, feeding and rumination patterns was recorded on a commercial dairy herd of 200 Holstein Friesian cattle using Afimilk SilentHerdsman. Milk fat, protein and lactose measurements plus four quarter milk conductivity measurements were recorded at every milking by a Fullwood Merlin milking robot. The cattle on the farm were monitored during the period March through to October 2017. 47 instances of mastitis were recorded.

Sensors within the milking robot measure the conductivity of the milk from each teat. Conductivity increases occur in advance of visible changes in foremilk or udder tissue. There are instances where sensors produce misleading readings. Combining conductivity and feeding/rumination data improves measurement reliability.

The milking robot alerts when milk conductivity increases above a nominal normal value. Normally, feeding/rumination time budgets are constant within +/- 20 %. The traces shown in **Error! Not a valid bookmark self-reference.** indicate a fall in feeding/rumination behavior with a mastitis infection. The conductivity increase is observed. This was confirmed by farm operatives as mastitis.

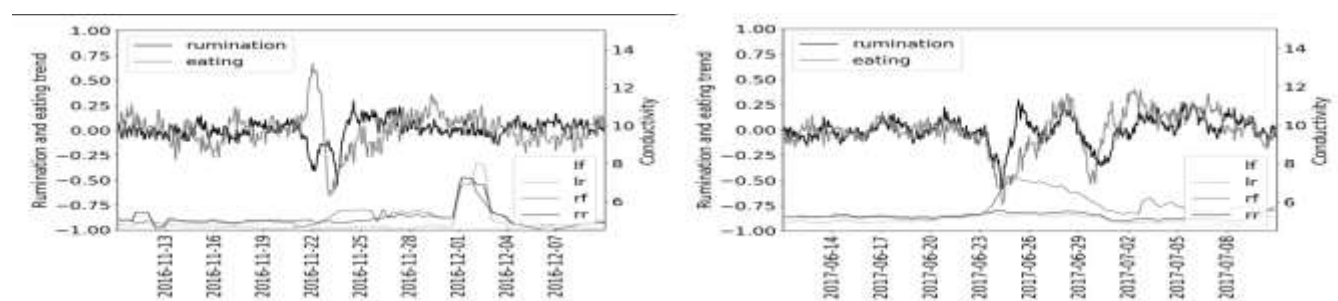
Figure 1 (left) below shows an example where the conductivity increased over

all four quarters. Collar derived welfare indicators (feeding/rumination) are stable. In the case of a mastitis incidence feeding and rumination would be expected to drop. The explanation is administration of a fertility treatment. All cattle treated this way displayed a rise in milk conductivity without behavioural changes. The corresponding trace (Normally, feeding/rumination time budgets are constant within $\pm 20\%$. The traces shown in **Error! Not a valid bookmark self-reference.** indicate a fall in feeding/rumination behavior with a mastitis infection. The conductivity increase is observed. This was confirmed by farm operatives as mastitis.

Figure 1 right) show drops in feeding/rumination and conductivity traces indicating a case of mastitis.

Normally, feeding/rumination time budgets are constant within $\pm 20\%$. The traces shown in **Error! Not a valid bookmark self-reference.** indicate a fall in feeding/rumination behavior with a mastitis infection. The conductivity increase is observed. This was confirmed by farm operatives as mastitis.

Figure 1: Illustration of Combination of Collar and Milking Robot Signals. Left: False Alarm Due to Fertility Treatment, Right: Genuine Alarm – Collar and Conductivity Signals Align



RESULTS

Milk conductivity data and feeding/rumination behavior was examined from 47 cases of mastitis. Contemporaneously, the farmer recorded welfare issues, instances of mastitis, treatment and recovery time. **Error! Reference source not found.** shows a summary of the trial findings. In all cases where feeding/rumination alerts coincided with milk conductivity changes, mastitis was also observed. In the majority of cases, feeding/rumination indicators alert prior to detection by the farmer.

Table 1: Comparative Analysis of Timing of Alert Relative to Observation

Measurement	Alert > 1 day before farmer	Alert before or same day as farmer
Feeding	74%	90%
Rumination	68%	84%
Conductivity	25%	48%

Fat/Protein	13%	38%
Lactose Drop	6%	25%
Milk time	19%	48%

ACKNOWLEDGMENT

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EFFECTIVENESS OF TEAT COVERAGE WITH POST MILKING TEAT DISINFECTANT APPLIED WITH A DIP CUP

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INTRODUCTION

Anecdotally, teat dipping has been considered the “Gold” Standard for effective coverage of the teat end and teat barrel. It was the first method of post milking teat disinfection introduced with the advent of the NIRD Five Point Plan in the 1960’s. However, it is considered time consuming and vacuum operated hand-held lance teat sprayers have become more common than dip cups in the milking parlour. Regardless of method of application the aim is to ensure complete coverage of the teat barrel and teat end with a suitable post-milking teat disinfectant. Good coverage will help ensure good bacterial kill of the teat surface but also (with a good emollient product) lead to teat skin that is soft and supple and which is able to withstand the rigours of milking. Two studies were carried out in 2013 (1) and 2014 (2 & 3) on teat spraying, the first with hand operated sprayers and the second with an automatic teat spray system. The latter provided a more consistent application and was more effective than manual teat spraying in teat barrel coverage. The purpose of this study was to measure post milking teat barrel and teat end coverage with the use of dip cups.

EVALUATION METHOD

Teat barrel and teat end coverage were assessed post application of the teat disinfectant product on ten farms, using the method described by Pocknee (1).

RESULTS

Teat end and teat barrel coverage are shown in the following three tables.

Table 1. Teat end and teat barrel coverage with disinfectant

	Average Number - Teat end coverage	Number for No teat end coverage	Number of missing quarters	Average % for Left teats	Average % for Right teats	Average % for Rear teats	Average % for Front teats	Average % for All teats
Study average	3.96	1.4	3.1	95.07	95.29	95.07	95.62	95.28
Minimum	3.92	0.00	2.00	88.30	90.26	90.63	87.93	89.21
Maximum	3.98	7.00	5.00	98.45	97.76	98.45	97.86	97.94

The results show a very narrow range in efficiency of teat dipping between farms. Over the ten farms on average between 89.2 and 97.4% of all teat barrels were coated in the post milking teat disinfectant, with an average of 95.3%. This contrasts remarkably with manual teat spraying (1) where the average was 50.3%, with a range between 19.8% and 83.4%. Teat ends, to all intents and purposes were covered with disinfectant, and there was no difference between the front and rear plane of teats.

Table 2. Percentage teat end coverage

	Rear Left	Front Left	Front Right	Rear Right	Average
Teat end only covered	99.5	99.6	99.9	99.8	99.7
No teat end coverage	0.5	0.4	0.1	0.2	0.3
No teat *	0.8	0.6	0.8	0.9	0.8

* three quartered cow and unit not applied

Table 3. Teat barrel coverage

	Rear Left		Front Left		Front Right		Rear Right	
	Back	Front	Back	Front	Back	Front	Back	Front
Average teat coverage (score out of 50)	46.9	46.5	48.5	48.1	47.6	46.9	48.4	48.2
No barrel coverage (number)	0.4	0.4	0.5	0.5	0.1	0.1	0.3	0.3
Average number of cows scored	112		112		112		112	

On average 10.3 ml disinfectant was used per cow, with a range from 7.7 to 13.1ml. This is almost identical to the rate often quoted.

CONCLUSION

Teat dipping provides a much greater efficacy of teat disinfection compared with manually spraying teats. Based on this evaluation study, teat dipping can rightly be described as the “Gold” Standard.

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MACHINE LEARNING PREDICTIONS OF HERD MASTITIS DIAGNOSIS

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Machine learning algorithms have been used in a variety of applications, ranging from the filtering of spam emails [1] to the suggestion of movies a Netflix user might next enjoy [2, 3]. These algorithms also have great potential within medical fields, approaching diagnostic problems much as a student doctor (or veterinarian) might; learning rules from data and applying them to new patients [4]. The use of machine learning in human health diagnostics has shown great promise, from the accurate classification of skin cancer [5] to outperforming internal medicine specialists in haematological diagnosis [6]. Machine learning within the field of cattle medicine includes the prediction of fertility events [7], high somatic cell counts [8], and the prediction of calving [9], however to date has not been utilised as a diagnostic support tool to make diagnoses at herd level.

The ability to correctly diagnose the putative origin of mastitis cases on farms is essential to implementing control strategies for prevention, as mastitis control elements for contagious origin mastitis are very different from environmental origin mastitis [10]. The nonlactating (“dry”) period has been shown to be at least as important as the lactating period in the epidemiology of intramammary infections [11]. The use of a categorical herd level mastitis diagnosis of either environmental dry period (EDP), environmental lactation period (EL) or contagious is one of the cornerstones of the dairy mastitis control plan (DMCP); an evidence based method of reducing mastitis levels [12].

In the preliminary analysis of this study, clinical and subclinical mastitis data from 42 farms was processed using supervised machine learning techniques, principally random forest [13]. The outcome variable to be classified by the algorithm was the diagnosis of mastitis origin as either contagious or environmental, with environmental being further subclassified as either dry period or lactation period origin. Statistical analysis is currently in progress, however preliminary analysis has demonstrated enormous potential of machine learning algorithms in the correct diagnosis of herd mastitis origin; with machine learning diagnostic accuracy of contagious vs environmental origin being around 95% when compared with expert diagnosis. Further research is currently underway aiming to analyse a significantly larger sample size.

There is great potential for machine learning algorithms to provide accurate decision support tools for practitioners, which can be used in conjunction

with the veterinarians' own clinical experience. The accurate diagnosis of mastitis origin can have great implications in the effectiveness of interventions in the control of mastitis. By providing practitioners with accurate decision support tools, there is great potential to augment and improve practitioners' ability to reduce the levels of mastitis on dairy farms.

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MAXIMISING MILKING EFFICIENCY: A PILOT STUDY OF CURRENT U.K. PARAMETERS AND FACTORS AFFECTING THE MILKING PROCESS.

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Milking efficiency impacts on all dairy businesses, regardless of herd size or husbandry system. Gains in milking efficiency will increase profitability by reduction of variable costs and increased dilution of the fixed costs of milk harvest. There may also be indirect effects on profitability through greater time availability for both cow and farm staff giving improvements in fertility, mobility and metabolic health. Different parameters have been utilised to describe milking efficiency in conventional milking systems. ‘Cows per hour’ and ‘milk per hour’ are commonly used, but the impact of production level and parlour size make these metrics inappropriate as comparators between farms. ‘Milk per stall per hour’ has been reported as better suited to inter-farm benchmarking. Targets of 50 kg per stall per hour (eight-hourly milking intervals) and 68 kg per stall per hour (twelve-hourly milking intervals) have been recommended in the USA (Reid, 2008). However, just 32% of randomly selected Israeli farms achieved 50 kg per stall per hour (Ginsberg, 2010).

A paucity of data describing UK milking efficiency highlights a necessity to investigate current milking parameters. This study was a pilot to establish an approach to collecting and processing data pertinent to milking efficiency. The primary objective was to compare different parameters to ascertain their relative usefulness to the UK situation. A secondary objective was to describe the current UK performance as judged by these different milking efficiency metrics. An additional aim was to examine on-farm variables for associations with milking efficiency. A survey was designed to collect data on herd size; milking times; milk yield; parlour type; parlour size; automatic cluster removal (ACR) settings; milking routine; and labour. Seventy-two respondents were recruited via social media (Twitter. San Francisco, USA); at dairy conferences (Total Dairy 2018, Stratford-upon-Avon; UK Dairy Day 2018, Telford); and directly via dairy clients of Advance Milking Limited. The data were processed to calculate ‘milk per hour’; ‘cows per hour’; ‘milk per stall per hour’; ‘milk per milking unit per hour’; and ‘milk per labour unit per hour’ (Microsoft Excel. Washington, USA). Linear regression models were generated to examine associations between on-farm variables and milking efficiency as described by each different metric (R v3.5.0. Vienna, Austria).

Milking efficiency was highly variable within each different metric. All efficiency metrics were correlated, but it was noticeable that the degree of correlation was not high. This suggests that different metrics are better suited to evaluating different types of dairy system. ‘Cows per hour’ is a useful metric for farms looking to improve throughput, but is of limited use in comparing herds with disparate mean cow yields. ‘Milk per hour’ is the metric most

closely related to profitability, but it is markedly affected by number of stalls and so not appropriate for comparing widely different parlour sizes. 'Milk per stall per hour' is the most appropriate benchmark between different sizes of parlour, but this metric underestimates the milking efficiency of 'swing-over' parlours, where 'milk per milking unit per hour' may be used. Therefore, it is apparent that no single parameter is 'best' at evaluating milking efficiency, rather different combinations of metrics are appropriate for evaluating different situations. 'Cows per hour' ranged from 39 to 378 cows (median: 114 cows). 'Milk per hour' ranged from 361 to 4549 kg (median: 1391 kg). 'Milk per stall per hour' ranged from 16-95 kg (median: 42 kg). 'Milk per milking unit per hour' ranged from 16 to 101 kg (median: 58 kg). This data showed that, of twice-daily milking herds, 6% exceeded 68 kg per stall per hour and 42% exceeded 50 kg per stall per hour. Of herds milking three times per day 46% exceeded 50 kg per stall per hour and 31% exceeded 68 kg per stall per hour. This calls in to question the application of 68 kg for twice daily herds as suggested by Reid (2008).

Rotary parlours have a positive association with 'milk per hour', accounting for an increase of 584 kg ($P < 0.001$), and 'milk per stall per hour' ($P < 0.05$). Mean daily cow yield was also positively associated with milking efficiency, with each 1 kg increase in cow yield giving an increase of 0.69 kg in 'milk per stall per hour' ($P < 0.001$) presumably due to a greater ratio of milking to non-milking occupancy with higher yielding cows. Use of ACR technology was associated with higher milking efficiency in models for all metrics. For farms with known ACR settings, 'milk per stall per hour' increased by 9.4 kg when detachment was triggered by milk flow greater than 300 ml per minute, compared to detachment at less than 300 ml per minute ($P < 0.05$).

As an invitational survey there was inevitable bias due to non-random selection, as well as limited ability to verify the data provided. The relatively small number of farms restricts the extent to which the data represents the UK situation, as well as limiting statistical power. True efficiency models would include data on milk price, udder health costings, and fixed and variable costs, but this was beyond the scope of this work. However, this pilot study has been successful in clarifying the role for different metrics of milking efficiency. Median values for 'milk per stall per hour' were lower than suggested targets, but a high degree of variation shows potential for vast improvement. Milking efficiency is positively associated with rotary parlours, mean cow yield and ACR detachment at flow rates greater than 300 ml per minute. Further work to expand the dataset will verify these findings and explore additional variables affecting milking efficiency.

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THE ROLE OF ZINPRO AVAILA®MINS IN MAMMARY GLAND HEALTH; A RESEARCH SUMMARY

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BACKGROUND

Mammary gland health is dependent on the effectiveness of the immune system, the ability of the mammary gland to involute, recover and regenerate between lactations and the integrity of the primary defences. A significant proportion of antibiotic use in dairy cows is linked to bacterial infections of the udder and routine use of antibiotics at drying off. In the UK, the government is targeting a 50% reduction in the use of intra-mammary high priority critically important antimicrobials and a reduction of 20% in the use of dry cow therapy by the year 2020. Nutritional interventions that lower the susceptibility to disease through improvements in epithelial integrity and immune function and help reduce the use of antibiotics would be advantageous. Here we present a summary of published research that demonstrates how supplementation with Zinpro's unique amino acid-metal complexed trace minerals can affect mammary health.

RESULTS

Summarised performance data from 12 trials where a proportion of the supplemental zinc was replaced with Availa®Zn showed an average reduction in SCC of 98,000 cells/ml ($P < 0.01$) (Kellogg *et al.*, 2004). Nayeri *et al.*, (2014) demonstrated a linear reduction in the milk SCC linear score of dairy cows when 75ppm of zinc sulphate was partially replaced with 16 and 40 ppm of Availa®Zn ($P = 0.13$). Similar reductions have been observed in other species including sheep (Kotsampasi, 2017, unpublished) and swine (Rapp and Morales, 2016). These improvements are associated with improved epithelial integrity, teat keratin formation, a reduction in oxidative stress, controlled inflammation and enhanced immune function.

Mastitis damages the integrity of the mammary epithelium and can lead to depression of milk production (Stelwagen *et al.*, 1994a,b) and transfer of bacterial cell walls into the blood circulation. In order to investigate the effect of Zinpro trace minerals on tight junction integrity Weng *et al.* (2018) used a heat stress model to disrupt the mammary epithelium and plasma lactose as a marker for epithelial integrity. Cooled and non-cooled cows were fed a total of 75ppm of supplemental Zinc. Control diets were 75ppm as Zinc hydroxychloride. In the treatment diets 40ppm of the zinc hydroxychloride was replaced with 40ppm of Availa®Zn. During the baseline period cows fed the Availa®Zn had significantly ($P < 0.05$) lower plasma lactose concentrations at -45 and -17 hours prior to the heat stress challenge, and upregulation of the tight junction protein gene E-Cadherin ($P < 0.09$), suggesting improved epithelial integrity. Tight junction integrity in other tissues can also affect milk

SCC. Kvidera *et al.*, (2017) showed that cows suffering from leaky gut induced by heat stress have elevated milk SCC ($P=0.07$).

Keratin found in the streak canal acts as a physical barrier, preventing microorganisms from entering the udder. It also has bactericidal and bacteriostatic properties that can cause lysis of gram positive bacteria. Spain *et al.*, (2005) fed dairy cows either diets containing no supplementary zinc, 800mg of supplementary zinc as zinc oxide or 400mg of zinc oxide and 400mg of Availa®Zn. Cows supplemented with Availa®Zn tended to produce significantly more Keratin ($P<0.10$).

Phagocytosis is the primary immunological defence against invading pathogens in the mammary gland. Osario *et al.*, (2016) fed two groups of cows a base diet containing identical levels of supplementary Zinc, Manganese, Copper and Cobalt, but in the treatment group 40ppm Zn, 20ppm Mn, 5ppm Cu and 1ppm Co was provided as Zinpro Availa®Mins. The diets were fed for 30 days prior to and 30 days post parturition. Feeding Zinpro Availa®Mins resulted in improvements in immune function characterized by increased phagocytic ($P=0.08$) and antioxidant ($P=0.01$) capacity postpartum.

Oxidative stress can suppress phagocytic capacity (Osario *et al.*, 2016), and result in immune system dysfunction (Sordillo and Aitken., 2009). Inflammation is necessary for normal immune function, overcoming infection and restoring homeostasis, but uncontrolled it can reduce reproductive and production performance. Using the same methodology as Osario *et al.*, (2016), (Batistel *et al.*, 2016) demonstrated that feeding Availa®Mins affected the production of biomarkers and the expression of genes characteristic of reduced inflammation and oxidative stress. Inflammation can be controlled by efficient removal of proinflammatory factors such as lipo-polysaccharide. Raised SCC are a useful indicator of inflammation. In a different experiment, cows supplemented with 40ppm Availa®Zn as part of 75ppm total supplementary Zinc reduced milk SCC at a faster rate following an induced LPS challenge than cows fed 75ppm of inorganic zinc (Horst., *et al.*, 2018)

CONCLUSION AND IMPLICATIONS

It has been demonstrated that improvements in mammary gland health can be realised through effective trace mineral nutrition. These improvements have the potential to reduce the routine use of antibiotics as well as the use of antibiotics for clinical and sub-clinical infections. Zinpro Availa®Mins have the added advantage of consequential improvements in animal performance, feed efficiency and hoof health. This reduced antibiotic approach to tackling udder health is aligned with current government strategy and consumer demand.

MILK QUALITY IMPROVEMENT INITIATIVE ON JERSEY

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Scientific evidence suggests that defects in dairy products can arise due to the presence of thermotolerant and psychrotrophic bacteria in raw milk.

Changes in product distribution patterns, growing export market and greater consumer expectations have resulted in a greater demand for high quality dairy products with a longer shelf life.

The Jersey Dairy quality team has always focused on improving product quality. In 2014, Jersey Dairy took the decision to further improve the raw milk quality from their 20 supplying farms and thermotolerant and psychrotrophic tests were introduced. Technical support for producers was organized incorporating a combination of producer meetings with monitoring and improving many husbandry practices at individual farm level. Twice monthly bulk milk tank analysis was initiated with advice and commentary provided after direct plating and identification of predominant bacteria in bulk milk samples. A bonus scheme to reward low levels of thermotolerant and psychrotrophic was also introduced.

Since the launch of the milk quality initiative, the quality of the raw milk has improved, final product quality was improved while shelf life of UHT, pasteurized milk, butter, cream and recipe based products has been significantly extended. Jersey offers a unique opportunity to assess the effectiveness of a milk quality initiative as all milk is collected, delivered and processed by a single source. This means the benefit can be quantified through the whole chain of production.

This initiative could potentially form a template for other milk quality programmes, combining technical support at a group and individual farm level, comprehensive milk quality testing and focused milk processor committed to improving milk quality.

UDDER HEALTH PARAMETERS FROM UK SENTINEL HERDS FOR 2017

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The AHDB Dairy Sentinel Herds project aims to monitor trends in clinical and subclinical mastitis over time. In 2016, 118 Sentinel Herds reflecting the geographical distribution of dairy farms in England, Wales and Scotland, were recruited with the criteria of 1) reliable electronic recording of clinical mastitis and 2) preferably monthly Individual Cow Somatic Cell Count recording (1). An additional six herds were recruited in 2017, to maintain numbers in case of 'wastage'. Participating farms provided data on clinical mastitis cases, and milk recording information, in electronic format. Two farms from the original 2016 failed to provide data. TotalVet software (www.total-vet.co.uk) was used to calculate 12 month averages for key udder health parameters. This poster reports 2017 figures and compares the results for the periods ending 31 Dec 2016 and 31 Dec 2017.

The key results for 2017 are summarised in Table 1. The changes between 2016 and 2017 are summarised in Table 2, for the 116 farms with robust data sets for both years. Key parameters for 2017 and 2016 are compared in Table 2. Bulk milk SCC increased by 10.9% over this period, while the majority of other udder health indicators improved. Mean clinical mastitis rate decreased by 11.4%, with a greater reduction in cases of lactation origin (14%) compared with dry period origin (8%).

Table 1 Key farm indices and udder health indicators 2017

Variable	N	Mean	Median	SE mean	Min	Max
Herd size	122	321	259	23.2	64	1490
Mean annual rolling 305 day yield (l)	116	8721	8844	163.4	4102	13797
Calculated bulk milk SCC (,000/ml)	115	172	157	7.8	60	517
Clinical mastitis (CM) rate (cows affected 100 cows/ year)	122	34.2	29.0	2.0	5	124.0
Quarter CM rate (/100 cows/ year)	122	37.6	31.0	2.2	5.0	132.0
Dry period origin CM rate (cows in 12)	122	0.8	0.6	0.1	0	7
Lactation origin CM rate (cows in 12)	122	1.9	1.8	0.08	0	6

Variable	N	Mean	Median	SE mean	Min	Max
Lactation new infection rate (%)	120	7.2	6.6	0.30	2	19
Dry period new infection rate (%)	120	15.9	14.6	0.7	4	39
Dry period cure rate (%)	120	78.4	79.6	0.9	47	100
Fresh calver infection rate (%)	120	17.3	15.9	0.7	4	45
% chronically infected	120	9.0	8.0	0.4	1	32
% > 200,000 cells/ml	120	16.3	16.0	0.6	5	43

Table 2 Key udder health indicators 2016 and 2017 for 116 farms

Variable	2017 Mean	2016 Mean	Change	% change
Calculated bulk milk SCC (,000/ml)	173	156	17	+10.9
Clinical mastitis (CM) rate (cows affected 100 cows/ year)	34.2	38.6	-4.4	-11.4
Dry period origin CM rate (cows in 12)	0.81	0.87	-0.06	-6.9
Lactation origin CM rate (cows in 12)	1.91	2.21	-0.31	-13.6
Lactation new infection rate (%)	7.3	7.9	-0.6	-7.6
Dry period new infection rate (%)	16.1	15.9	0.2	1.3
Dry period cure rate (%)	78.3	76.7	1.6	2.1
Fresh calver infection rate (%)	17.5	17.7	-0.2	-1.1
% chronically infected	9.1	10.0	-0.9	-9.0
% > 200,000 cells/ml	16.5	17.7	-1.2	-6.8

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COW MILK BIOREPOSITORY: A TOOL TO INVESTIGATE CLINICAL AND PRE-CLINICAL DISEASES UNDER CONTROLLED CONDITIONS

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SUMMARY

Diagnostic and screening tools are becoming more and more important in the field of animal productions. This topic is particularly relevant, not only for business-related issues linked to dairy industry, but with respect to the One Health concept. Being able to rapidly characterize easily bioavailable specimens, such as milk, for the detection of cow-husbandry-related conditions means providing the tools for a prompt and effective intervention. This is particularly true, for example, in the case of mastitis and antibiotic resistance.

Consequently, a longitudinal well-characterized sample collection from a controlled research environment provides the basis for analyzing health conditions/pathologies in relation to time. At the University of Reading (UoR) we have recently started such sample collection.

INTRODUCTION

The study of different pathologies in the field of cow husbandry can be very difficult, particularly if it is necessary to target the pre-clinical phase. Pre-clinical mastitis has been investigated using animal models obtained with experimental bacterial infection [1, 2]. Although these efforts represent a good start for the comprehension of the physiology and the timing of the infection, are still far from the real condition where animals can be exposed to several different pathogens and produce different indirect (secondary) biomarkers.

In order to achieve this task, the construction of a cow milk biorepository was initiated, based on the collection of individual samples of milk on a weekly basis from the Centre for Dairy Research (CEDAR) at UoR for a period of four months.

MATERIALS & METHODS

Sample collection has been done at CEDAR where around 500 Holstein cows are milked daily and monitored for the presence of clinical signs of mastitis as visible signs in the udder or presence of clots in milk.

Milk sampling for the biobank has been carried out from July 2018 to October 2018 on a weekly basis allowing the storage of individual milk samples from 450 to 500 cows. Samples have been collected through the collector of a 50 places rotary parlour (Dairymaster). One aliquot of 1.5-2 ml of each sample has been collected in a screw-capped 2-ml cryovial (Fisherbrand™, cat n°10-500-26), frozen on the spot in dry ice and stored at -80.

Moreover, every sample is analysed for somatic cell count (SCC), milk volume, protein content and fat content.

RESULTS

To date, around 6000 samples have been collected over a period of 14 weeks. Milk yield showed a very dynamic range from 8.3 kg/day to 63 kg/day (July 2018). The SCC number detected in each individual sample ranged from a minimum of 10,000 to a maximum of ~4,000,000 per ml, with 350 cows under the threshold limit of 200,000.

The incidence of clinical mastitis during the month of July 2018 was 22 cases out of 483 sampled cows.

DISCUSSION

The purpose of this new biobank tool is to provide the possibility to study the evolution of diseases and health conditions over time in an easily collected and biologically informative specimen, such as milk.

The dataset obtained so far confirms that an SCC number higher than 200,000/ml is related to a higher probability of mastitis infection in at least one quarter. However, as shown in the results section, an average number of 150 cows presented with an SCC number higher than 200,000 but only 22 were diagnosed with clinical mastitis. This underlines the necessity to develop more accurate screening methodologies.

This biobank offers the possibility to study each condition before clinical events and, in the case of mastitis, provides the basis to study around 100 cases (with adequate controls) during a period of four months in their pre-clinical phase.

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EVALUATION OF SELECTIVE CULTURE-BASED MEDIA FOR DETECTION AND DIFFERENTIATION OF BACTERIA ASSOCIATED WITH BOVINE CLINICAL MASTITIS

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The present study aimed to evaluate the accuracy of selective culture-based media (hereinafter, “test kit”) for detection of bacteria associated with bovine clinical mastitis (CM) in dairy cows against a “gold standard” consisting of bacteriological culture and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF).

Milk samples from CM cases (i.e. presence of clots, flakes or serous milk, with or without additional local and systemic signs of disease) were collected aseptically by trained staff from seven dairy farms in Scotland. Farms were selected based on location, ease of access and willingness to cooperate in the study. Samples were frozen on farm (-20 °C) and cultured within 4 weeks from sampling. Samples were processed using standard laboratory methods¹ and the new test kit. The test kit comprises two distinct sectors of selective medium: one selective for Gram negative (GN) and one selective for Gram positive (GP) organisms. Media were inoculated with 0.01 ml per side using calibrated sterile plastic loops. Concurrently, 0.01 ml of the same milk sample was inoculated onto both Sheep Blood Agar 5% and MacConkey agar number 3 plates (E & O Laboratories Limited, Bonnybridge, Scotland). Kits and plates were incubated at 37°C in aerobic conditions and were examined after ca. 24 and 48 hours. Kits and plates with no visible colonies were considered negative for mastitis-associated pathogens. Plates that contained three or more morphotypes were considered contaminated¹ and excluded from the analysis. For the remaining plates, each morphotype was counted semi-quantitatively and assigned to one of four categories (1-10 colonies, 10-50, 51-200, >200). Isolates were subsequently sub-cultured onto half of a sheep blood agar plate for purification. From each pure culture, a colony was selected and grown in 2 ml of Brain and Heart Infusion (BHI) broth for 24 hours at 37 °C in aerobic conditions. The isolates were preserved with 15% of glycerol in cryovial at -80 °C, and submitted to an external laboratory for species identification by MALDI-ToF.

The definition used to consider a quarter as infected was the lenient definition, i.e. any culture-positive result². According to this definition, from 156 samples, 23 were contaminated, and 3 yielded growth but without pathogen identification by MALDI-ToF, therefore excluded from the analysis. From the remaining samples, 14 showed no growth and 116 samples yielded growth with a total of 135 identified isolates, with 97 from samples with one morphotype and 38 from 19 samples with two morphotypes on culture. The

most common species was *Escherichia coli* (37.5%), followed by *Streptococcus uberis* (15.4%) and non-aureus staphylococci (11.8%). The accuracy of the test is of 87%. The test had a Sensitivity (Se), Specificity (Sp), Positive predictive value (PPV) and Negative predictive value (NPV) for GP bacteria of 82%, 77%, 76%, and 83% respectively. For GN bacteria, Se, Sp, PPV, and NPV were respectively 83%, 94%, 93% and 87%. For *E. coli* identification a Se, Sp, PPV and NPV were respectively 84%, 97%, 96% and 91%.

This test is very easy to perform and to interpret with results available within 24 hours. The test is good on detecting both GP, GN and *E. coli*, with less than 20% of false negatives. Though, has some limitations detecting cows that would not need AB treatment, i.e. GN and no growth, with a fair amount of false GP. Regarding GN bacteria in general and particularly *E. coli*, just a small amount false positives, 6%, and 3% respectively, will be misdiagnosed as GP or no growth.

In conclusion, the test kit has the potential to inform selective treatment for CM withholding antimicrobial treatment from cases where Gram-negative bacteria are cultured.

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