

2016

BRITISH MASTITIS CONFERENCE

Organised by

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The University of
Nottingham

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Topics:

- Milk Amyloid A
- Selective dry cow therapy
- Research updates
- Automated mastitis control technology
- IDF 2016
- On-farm culturing

Wednesday 2nd November 2016

Ricoh Lounge, Worcester Rugby Club,
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GENERAL INFORMATION

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CHAIRMAN'S INTRODUCTION

Welcome to the 2016 British Mastitis Conference.

The Organising Committee has been working since last year's conference to bring together an expert group of speakers, not only from the UK, but also Denmark, France and Brazil. We trust that you will find the programme both thought provoking and stimulating.

2015 saw the linking of the BMC with the inaugural meeting of the European Mastitis Research Workers. Based on its success, the EMRW meeting will follow BMC, but this year over two days. We welcome our international colleagues who are attending this conference.

Our first paper looks at MAA (Methyl Ameloid A) and its role in dry cow therapy. This is followed by a paper on the practical experiences with selective dry cow therapy.

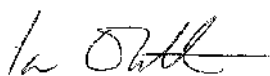
Building on the previous success, and endorsement by delegates, we have selected four posters from the Knowledge Transfer section for oral presentation. The four papers are followed by an opportunity for delegates to debate with the presenters.

After lunch, we will turn our attention to the latest developments in automated parlour technology to detect mastitis. This will be followed by a change in the original programme with a review of the September IDF Mastitis Conference held in France. The conference will be closed with a paper on the practical application of on farm culturing – its benefits and the potential pitfalls.

This year sees an excellent selection of high quality poster submissions – a total of 14. 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.

As always 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, with a record eleven supporting the conference. This year our sponsors are: Bimeda (Gold), Elanco (Gold), Kilco (Gold), MSD Animal Health (Gold), Fullwood Ltd (Silver), Milkrite InterPuls (Silver), Zoetis (Silver), Boehringer-Ingelheim (Bronze), Norbrook (Bronze), Ambic (Bronze), DeLaval (Bronze) and NW Resources Ltd (Bronze). As always the event could not happen without able administration, provided by Karen Hobbs and Anne Sealey at *The Dairy Group*.

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



Ian Ohnstad, British Mastitis Conference Chairman
The Dairy Group

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	Update on MAA & how applied to dry cow therapy.	Cyril Crosson Bioteck Lait, France
10:25	Practical experiences with selective dry cow therapy.	Peter Orpin Park Veterinary Group, Leicester, UK
10:55	Questions and Discussion	
11:10	COFFEE and POSTERS	
	Research updates (also presented as posters)	Elizabeth Berry BCVA, UK
11:40	Cost-effectiveness of on-farm culture for the treatment of clinical mastitis.	Peter Down University of Nottingham, UK
12:00	Identification of potential markers in blood as a risk factor for occurrence of bacteriologically positive quarter milk samples in dairy cows during the transition period.	Fernando Souza Universidade de São Paulo, Brazil
12:20	Impact of the AHDB Dairy Mastitis Control Plan.	Katharine Leach QMMS, Wells, UK
12:40	<i>'It's the dollar value... isn't it?'</i> Form, function and efficacy of veterinary advice for farmer behaviour change: a qualitative investigation.	Alison Bard University of Bristol, UK
13:00	LUNCH and POSTERS	
14:10	WELCOME BACK AND VOTING ON POSTERS	
	Session Three	Brian Pocknee DHC, UK
14.15	Latest developments in automated parlour technology to detect mastitis.	John Baines Fullwood Ltd, UK
14.45	Update from 6 th IDF Mastitis Conference.	Elizabeth Bury BCVA, UK
15.15	Practical application of on farm culturing.	Michael Farre SEGES Dairy Research, Denmark
15.45	Questions and Discussion	
16:00	POSTER AWARD and CLOSE	
16:05	TEA and DEPART	

Titles of Papers and Presenters

Scientific programme

Session One

Update on MAA & how applied to dry cow therapy. 1 – 7
Cyril Crosson, Bioteck Lait, France

Practical experiences with selective dry cow therapy. 9 – 22
Peter Orpin, Park Veterinary Group, Leicester, UK

Research Update Session (also presented as posters)

Cost-effectiveness of on-farm culture for the treatment of clinical mastitis. 23 – 24
Peter Down, University of Nottingham, UK

Identification of potential markers in blood as a risk factor for occurrence of 25 - 26
bacteriologically positive quarter milk samples in dairy cows during the transition
period.
Fernando Souza, Universidade de São Paulo, Brazil

Impact of the AHDB Dairy Mastitis Control Plan. 27 – 28
Katharine Leach, QMMS, Wells, UK

'It's the dollar value... isn't it?' Form, function and efficacy of veterinary advice 29 – 30
for farmer behaviour change: a qualitative investigation.
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Latest developments in automated parlour technology to detect mastitis. 31 – 38
John Baines, Fullwood Ltd, UK

Update from 6th IDF Mastitis Conference. 39 – 45
Elizabeth Bury, BCVA, UK

Practical application of on farm culturing. 47 - 49
Michael Farre, SEGES Dairy Research, Denmark

Titles of Posters and Authors

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

Immune response induced by a vaccine assembly of recombinant proteins of *Staphylococcus aureus* and DNA vaccine: a study in a mouse model

D.L.S.O. Rosa¹, F.N. Souza^{2,3*}, L.A.F. Andrade⁴, E.M.S. Dorneles², T.M. Alves², E.M. Ramos Sanchez⁵, C.Y. Ozaki⁵, H. Goto⁵, M.A. Gidlund⁶, A.P. Lage², M.A.V.P. Brito⁷, A.M.M.P. Della Libera³, F.G. da Fonseca⁴, M.B. Heinemann⁸, M.M.O.P. Cerqueira¹

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Identification of potential markers in blood as a risk factor for occurrence of high milk somatic cell counts in dairy cows during the transition period

K.R. Santos¹, M.G. Blagitz¹, F.N. Souza^{1*}, C.F. Batista¹, H.G. Bertagnon¹, S.A. Diniz², M.X. Silva², J.P.A. Haddad², A.M.M.P. Della Libera¹

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Process control in platform mounted teat disinfection systems

Ian Ohnstad¹, Colin Kingston², Richard Hiley², Matthew Barker² and Mark Cinderey²

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Providing producers with information to make informed decisions on SDCT

Kate Cross, Cattle Information Services, Telford, UK

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Acute phase proteins profile of milk in multiple pathogen infections

F.C Thomas¹, I. Nianjani², H.Haining³, P.D Eckersall⁴

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Pegbovigrastim: A History of the future

John Cook

Ruminants Technical Consultant UK, Elanco Animal Health, Lilly House, Priestley Road, Basingstoke, Hampshire, RG24 9NL, UK

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Relationship between milk cathelicidin abundance and microbiologic culture in clinical mastitis.

M.F. Addis¹, V. Bronzo², G. M. G. Puggioni¹, C. Cacciotto¹, V. Tedde¹, D. Pagnozzi¹, C. Locatelli², A. Casula², G. Curone², S. Uzzau¹, P. Moroni^{2,3}

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Titles of Posters and Authors - continued

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

Diagnostic performance of a milk cathelicidin ELISA for mastitis detection

M.F. Addis¹, V. Tedde¹, G.M.G. Puggioni¹, S. Pisanu¹, A. M. Roggio¹, D. Pagnozzi¹, S. Dore², S. Lollai², V. Bronzo³, C. Locatelli³, A. Casula³, N. Rota³, E. A. Cannas², P. Moroni^{3,4}, S. Uzzau¹

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Can the IceQube be used as an early alerting system for clinical mastitis?

R.J. Tavernor^{1,3}, V. Thorup², R. Boyce², M. Rose³

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Development of a lateral flow point of care test for the rapid detection and measurement of haptoglobin in bovine milk

N. Brady, E. L. O'Reilly & P. D. Eckersall

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH, UK

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

(presenting author underlined)

Cost-effectiveness of on-farm culture for the treatment of clinical mastitis.

P.M. Down¹, A.J. Bradley^{1,2}, J.E. Breen^{1,2}, M.J. Green¹

¹University of Nottingham, School of Veterinary Medicine & Science, Sutton Bonington, Loughborough, LE12 5RD, UK; ²Quality Milk Management Services Ltd, Cedar Barn, Easton Hill, Easton, Wells, BA5 1DU, UK

23 – 24

Identification of potential markers in blood as a risk factor for occurrence of bacteriologically positive quarter milk samples in dairy cows during the transition period.

K.R. Santos¹, M.G. Blagitz¹, F.N. Souza^{1*}, C.F. Batista¹, H.G. Bertagnon¹, S.A. Diniz², M.X. Silva², J.P.A. Haddad², A.M.M.P. Della Libera¹

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25 – 26

Impact of the AHDB Dairy Mastitis Control Plan.

A.J. Bradley^{1,2}, J.E. Breen^{1,2}, K.A. Leach¹, D. Armstrong³, M.J. Green²

¹Quality Milk Management Services Ltd, Cedar Barn, Easton Hill, Easton, Wells, BA5 1DU, UK; ²School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, LE12 5RD, UK; ³AHDB Dairy, Stoneleigh Park, Kenilworth, CV8 2TL, UK

27 – 28

'It's the dollar value... isn't it?' Form, function and efficacy of veterinary advice for farmer behaviour change: a qualitative investigation.

Bard, A.M.¹, Main, D.C.J.¹, Haase, A.², Roe, E.³, Whay, H.R.¹, Reyher, K.K.¹

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FURTHER INFORMATION

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

What does NMC do?

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

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reducing mastitis and
enhancing milk quality*

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- To provide leadership on milk quality issues within the industry
- To participate and learn about mastitis and milk quality developments at NMC meetings
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PAPERS

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UPDATE ON MAA & HOW APPLIED TO DRY COW THERAPY

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SUMMARY

Antibiotics used in animal production contribute to the development of antimicrobial resistance and are subject to controversy in public opinion. In this context, some countries around the world advocate a cautious and rational antibiotic use in veterinary medicine. A selective dry cow therapy programme would enable a significant reduction in the use of antibiotics in dairy production. Milk Amyloid A (MAA) is suggested in several studies as a biomarker of both clinical and subclinical bovine mastitis. We conducted a field study to evaluate the efficiency of the measurement of MAA when used to make selective antimicrobial treatment decisions at quarter level on cows at drying off. We developed an algorithm to identify an intramammary infection (IMI) at mammary quarter level at the end of lactation. The algorithm is based on the MAA concentration and somatic cell count (SCC) results gathered prior to drying off and based on collected cow/milk data during lactation. The sensitivity and specificity of the algorithm were high, respectively 94.5% and 93.0% and the positive (PPV) and negative (NPV) predictive values were respectively 96.3% and 89.9%. The use of our algorithm in a selective dry cow therapy programme at the quarter level would allow a significant reduction in the use of intramammary antibiotics with confidence. In our study, its application would have reduced the use of antibiotic treatments by 29%.

INTRODUCTION

Antibiotic use in agriculture creates a selective pressure on bacterial populations and contributes to the development of antimicrobial resistance (12,6). They are also the subject of controversy in public opinion notably regarding the human health. In this context, some countries around the world advocate a cautious and rational antibiotic use in agriculture. As part of mastitis control and prevention at drying off, most dairy cow producers in North America and in many European countries treat all quarters of all cows with an intramammary antibiotic therapy at the end of lactation. An alternative approach to this practice is a selective dry cow therapy programme which would enable a significant reduction in the use of antibiotics in dairy production.

The Serum Amyloid A proteins in milk also called Milk Amyloid A (MAA) are suggested as a biomarker of both clinical and subclinical bovine mastitis in several experimentally or naturally acquired mastitis studies (1-4,9). An increase in concentration of MAA from infected quarters or udders is

observed whatever the pathogen causing mastitis, major, minor, environmental, contagious, Gram positive or negative. This increase in concentration from infected quarters is detected early in experimentally acquired clinical or subclinical mastitis studies, few hours after a challenge using a minor or a major pathogen strain (8,5,2). A fast return towards baseline levels after bacterial clearance is observed (5). MAA is also suggested as an indicator of mammary gland recovery. No effect of extramammary pathologies on MAA concentration is observed in the study of Nielsen and his collaborators (7). High diagnostic values to screen natural clinical or subclinical mastitis are reported (1,10,11). The sensitivities and specificities are all above 90%.

We conducted a field study to evaluate the efficiency of the measurement of MAA when used to make selective antimicrobial treatment decisions at mammary quarter level on cows at drying off. We developed an algorithm to identify an IMI at mammary quarter level at the end of lactation. The test characteristics of the algorithm were calculated and its NPV and PPV were estimated. Our algorithm was compared with the SCC at the quarter level recording prior to drying off and with the SCC recording in the last DHI tests of lactation.

MATERIALS & METHODS

Individual mammary quarter milk samples from 112 cows, originating from low bulk tank SCC (<250,000 cells/mL) dairy herds (n=6), were collected between two to seven days prior to drying off (n=443) and after calving (n=339), starting from two weeks until six weeks. All milk samples were cultured for bacterial detection and identification and they were analyzed for SCC and for MAA concentration using a patented commercial ELISA (Milk Amyloid A-MAA Assay Kit, cat. no. TP-807; Tridelta Development Ltd, Maynooth, Ireland). Cow/milk data throughout lactation were collected.

We performed a selective dry cow therapy at quarter level based on MAA results. The mammary quarters from cows with an MAA concentration ≥ 1 $\mu\text{g/mL}$ (n=257) were treated with an intramammary antibiotic therapy and were infused with a teat sealant. The other quarters (MAA < 1 $\mu\text{g/mL}$) were only infused with a teat sealant and were not treated with an antibiotic therapy (n=94) or they were treated by antibiotic therapy and infused with a teat sealant (n=92).

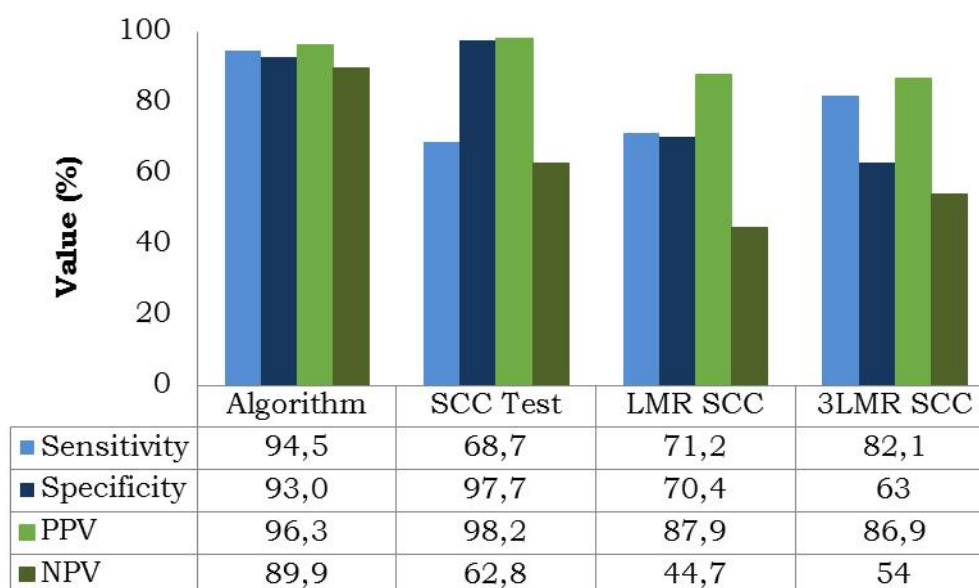
The developed algorithm to screen for an IMI at quarter level in cows prior to drying off was based on the gathered MAA and SCC results and on collected cow/milk data. Further details are not given for reasons of commercial sensitivity. The test characteristics of the algorithm and the SCC tests were calculated using bacteriological culture/detection as a gold standard. Their NPV and PPV were estimated using the formulas based on Bayes' theorem. A threshold of 100,000 cells/mL for primiparous cows or of 150,000 cells/mL for multiparous cows to identify an IMI was used for the SCC test at the quarter level recording prior to drying off (SCC Test) and for the SCC

recording in the last DHI test (LDHI SCC). The four mammary quarters of one cow were healthy considered if the cell count was below these thresholds at the last DHI test and if no clinical mastitis was recorded the last four months prior to drying off. Otherwise, the quarters were infected considered. A threshold of 100,000 cells/mL was used for the SCC recording in the last three DHI tests (L3DHI SCC) of lactation. For this test, the four mammary quarters of one cow were healthy considered if any cell count was over the threshold in the last three tests of lactation. Otherwise, the quarters were infected considered.

RESULTS & DISCUSSION

The sensitivity and specificity of the developed algorithm were both high, respectively 94.5% and 93.0% and the PPV and NPV in our study population were respectively 96.3% and 89.9%. By contrast, the sensitivities and the NPVs of the three SCC tests and the specificity of the SCC tests recording in the last DHI tests of lactation were low (Figure 1).

Figure 1. Test characteristics, positive (PPV) and negative (NPV) predictive values of the algorithmic and SCC tests applied to identify an IMI at the end of lactation



A threshold of 100,000 cells/mL for primiparous cows or of 150,000 cells/mL for multiparous cows was used for the SCC test at the quarter level recording prior to drying off (SCC Test) and for the SCC recording in the last DHI test (LDHI SCC). The four mammary quarters of one cow were healthy considered if the cell count was below these thresholds at the last DHI test and if no clinical mastitis was recorded the last four month prior to drying off. A threshold of 100,000 cells/mL was used for the SCC recording in the last three DHI tests (L3DHI SCC) of lactation. The four mammary quarters of

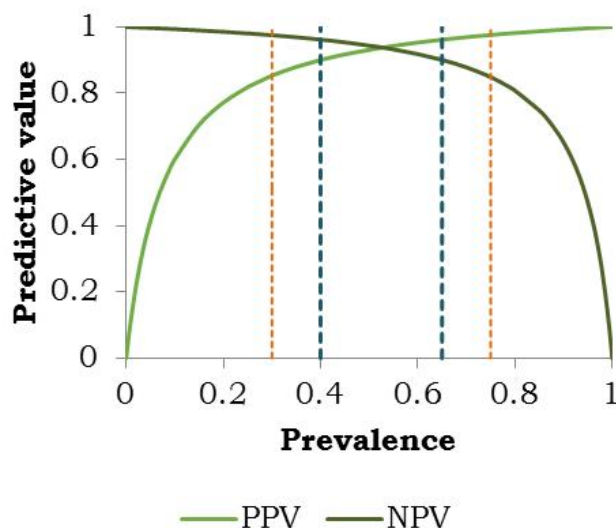
one cow were infected considered if any cell count was over the threshold in the last three tests of lactation.

As a result, our algorithm could be used to make selective dry cow treatment decisions at quarter level with the confidence that very few infected mammary quarters would be missed and that high proportion of the healthy mammary quarters would be not treated with antibiotic therapy. In contrast, the SCC at the quarter level recording just prior to drying off and the use of the SCC recording in the last DHI tests of lactation seem unsuitable as part of this selective programme.

The prevalence of infected quarters at the end of lactation in our study was 65.5%, according to our bacteriological culture results. The most frequently isolated pathogens were minor pathogens (94%), in descending order, coagulase negative staphylococci (CNS) (65%), followed by environmental Gram-positive pathogens *Corynebacterium* spp. (13%) and environmental streptococci (10%), predominantly *Aerococcus viridans* (7%). Some quarters were infected by two pathogens (7%). The effect on SCC is accepted to be generally limited or nonexistent for CNS as a group (13). This nonexistent effect would explain the low sensitivity obtained in our study for the tests based on SCCs. In contrast, our results indicate that the sensitivity of our algorithm was high. The algorithm was developed from the SCC level and from the MAA concentration level. Therefore, taking into account the MAA concentration seems to enable us to detect mammary quarters infected by CNS at the end of lactation. The possible use of MAA concentration levels in milk samples to detect mammary quarters infected by coagulase-negative staphylococci has been previously described (4,9).

The algorithm's predictive values were both above 90% when estimated in herd populations where the proportion of mammary quarters with an IMI at drying off ranged from 40% to 65%. Similar but more moderated results were obtained for a prevalence ranging from 30% to 75% (Figure 2).

Figure 2. Positive predictive value (PPV) and negative predictive value (NPV) of the algorithm when used to diagnose an IMI at mammary quarter level at drying off in cows from herds with low bulk tank SCC (<250,000 cells/mL) and for prevalence of IMI ranging from 0 to 100%



The vertical blue dashed lines indicate the prevalence range (40-65%) for which the PPV and the NPV both are above 90%. The yellow lines indicate the prevalence range (30-75%) for which the PPV and the NPV both are above 85%.

Consequently, in these various herds, the proportion of quarters truly infected with a negative algorithmic test sentence that would not receive an intramammary antibiotic treatment would be low ($1 - \text{NPV} < 10\%$) and few quarters truly healthy with a positive test sentence ($1 - \text{PPV} < 10\%$) would be treated with an antibiotic therapy.

Moreover, we didn't observe any clinical and subclinical mastitis six weeks after calving for quarters with a negative algorithmic test sentence that were not treated with antibiotics and were only infused with a teat sealant. These quarters have achieved success in the treatment and prevention of IMI over the dry period. By using our algorithm in our cow population, we would have reduced the antibiotic use by 29 % and also achieved the quantitative objectives of reducing antibiotic use advocate by the French ecoantibio2012-2017 plan.

CONCLUSION

We developed an algorithm to screen for an IMI at quarter level in cows prior to drying off. This algorithm utilizes the SCC result and the MAA concentration both measured in individual quarter milk samples and cow/milk data during lactation. Its usage in a selective dry cow therapy programme at the quarter level enables a significant reduction in the use of

intramammary antibiotics in dairy production with confidence. In our study population, its application would have reduced the use of antibiotic treatments by 29%.

In France since September 2015, we propose a service at drying off for dairy farmers based on our algorithm in order to they reduce the use of intramammary antibiotics treatments. The gathered data (MAA concentration, SCC, milk/cow data, bacterial identification/quantification) are used to grow, to reinforce our database that is used to support our algorithm.

ACKNOWLEDGEMENTS

This work was financed by a grant from the CNIEL (French National Center for Interprofessional Dairy Industries). Our group thanks Tridelta Development Ltd for their support in our R&D activities on MAA and the participating dairy producers.

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NOTES

PRACTICAL EXPERIENCES WITH SELECTIVE DRY COW THERAPY

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SUMMARY

Blanket Dry Cow Therapy(BDCT) has been widely accepted by UK Dairy farmers since the adoption of the 5 point plan. The use of antibiotics in non-infected cows has brought into focus with concerns regarding antibiotic resistance. Selective Dry Cow therapy(SDCT) has recently gained momentum in the UK with mixed results. This paper describes the practical experiences in a veterinary practice in Leicestershire and highlights the experience of one successful proponent of SDCT. The experience in practice has shown that successful SDCT requires detailed training and significantly improved dry off techniques to avoid severe mastitis or mortality.

A Survey Monkey review of 291 farmers' attitudes towards SDCT is discussed. The survey revealed that 80% of farmers believed that reducing antibiotic usage would be good for their livestock but 54% feared that SDCT would result in death or mastitis without antibiotic use. 17% had experienced severe mastitis and death of cows after infusion of teat sealant alone. 50% of farmers would recommend SDCT to another farmer. However the Net Promoter Score revealed there were more detractors (51%) than promoters (26%) indicating that without external support and encouragement the program may fail if relying on farmer recommendation. Division of the farmers into 3 groups-Proactivists, Unconcerned and Disillusionists revealed that the requirements to engage each group differed. Proactivists were interested in training and vet support whereas the other two groups required more convincing that SDCT would work and the benefits outweighed the risks. The discussion focuses on the next steps to consider if SDCT is to be successfully expanded in the UK taking into account the relative importance and beliefs of the farmers.

INTRODUCTION

BDCT has been widely accepted by UK Dairy Farmers with an estimated 99% of dairy farmers adopting this approach (Berry and Hillerton 2002). In Holland analysis of data indicates that 49% of total antibiotic use in Dairy herds was accounted by Dry Cow Therapy (DCT)(SDa). The O'Neill report (O'Neill.J.2016) commissioned by David Cameron made clear recommendations relating to antimicrobial use. A key action was for each country and sector to have "10 year targets to reduce unnecessary antibiotic use in agriculture". Irrespective of any considerations regarding the contribution of DCT to antimicrobial resistance the decision to introduce a

more targeted approach to DCT would be highly desirable for the dairy industry. Selective Dry Cow Therapy (SDCT) is defined as the selecting cows on an individual basis for antimicrobial therapy at drying off.

Work conducted in the UK by Bradley, Green and Huxley paved the way to more attention to the dry period and its importance in mastitis control (Bradley et al (2002,2003). The potential benefits of selective dry cow therapy were demonstrated by UK research almost 15 years ago (Huxley et al 2002). Further work by Bradley (2010) on SDCT approaches showed that different SDCT approaches could be applied according to the major pathogens causing mastitis. The use of teat sealant alone was more effective than in combination with cephalonium DCT at reducing the risk of gram negative mastitis in the subsequent lactation. BDCT may be more justified in herds with higher gram positive loads.

Specific guidelines on the selection criteria suitable for antibiotic DCT have been given (RUMA 2015, Biggs et al 2016, Roberts, 2014) and there is a continued debate as to the criteria for selection and herd and individual risks that may influence the decision at cow level. The approach that is developing within the UK is broadly a decision made based on individual somatic cell counts, teat condition and levels and type of bacteria present in the herd. Most commonly cows are selected for teat sealant only infusion if the last 3 cell counts prior to drying off are below 200,000 and the farmer defines the cow low risk of mastitis. The complexities of the pathogen load and herd level risk factors may not be as uniformly applied and requires expert veterinary interpretation and consideration.

The change from BDCT to SDCT requires a totally different approach by the farmer in terms of records, use of data, time invested in the procedure and infusion techniques. Anecdotally early use of teat sealant alone on a significant number of farms has resulted in severe mastitis and death and this has reduced the appetite for adoption of SDCT. This paper seeks to explore the practical steps that can be taken to help increase SDCT successfully based on practical experiences within the veterinary practice and survey data collected from UK farmers on their attitudes to SDCT.

PRACTICAL EXPERIENCES

Park Vet Group Experience

SDCT was utilised within the Park Veterinary Group when Orbeseal was first launched over 13 years ago. Initially the product was licensed for single use in 2002 and then this progressed to combination use with antibiotic DCT in 2005. Several early adopter progressive farmers in the practice utilised SDCT approaches with teat sealant alone being applied in low cell count cows. The results however were not successful. Several farmers experienced severe mastitis or more typically sudden death after infusion. These anecdotal reports impacted on our perception of the strategy resulting in a

loss of confidence in our recommendation of SDCT. The vets with the practice became much more guarded in their advice and unless we could be assured that the farmer was willing to adopt a high level of hygiene at the point of infusion we would advise BDCT. The reputational damage by association with a failed procedure had a significant emotional effect on both the veterinarian and the farmer.

Over the last 15 years we have conducted a number of extension meetings on mastitis including on farm demonstrations of the technique of infusion using outside experts (Andrew Bradley, Zoetis veterinarians). The adoption of SDCT was patchy but improving.

In 2015 Arla conducted a widespread knowledge transfer program on SDCT delivered by Kite Consulting. The meetings were well accepted and the practical demonstration of the technique of infusion has given more farmers the encouragement to re-engage with SDCT. Naturally if the milk processor supports and encourages the process this also provides an external incentive to commit to the program. Each Arla farmer, at the time of the farm assurance assessment, had to ask his vet to sign a document relating to the discussion and implementation of SDCT on farm. The more progressive farmers who believed in the concept and were willing to ensure that all staff followed the structured program of SDCT appear to have adopted the program well. There are limitations to adoption on certain of our farms (hygiene, levels of SCC, mastitis, operator skill) which do block progression from BDCT to SDCT. Ultimately the vet must have confidence that the strict methodology and approach required for SDCT has to be applied on farm for it to be a success. Partial hygiene approaches result in failure.

The plans for the next 2 years are to further assist farmers with their decisions to commit to SDCT by offering one to one training using a vet tech and directing farmers to the SDCT video on the website for reference. Vets will continue to assist farmers with cow selection with regular Interherd reporting. Increased surveillance for gram positive pathogens is important in the herds practising SDCT as we have experienced *Staph aureus* appearing in herds that have self-selected to reduce overall antibiotic DCT usage.

Shorn Hill Farm

Martin Beaumont of Shorn Hill farm is one of our more progressive dairy farmers with high and improving performance and standards every year.

The herd is a 275 cow all year around calving herd producing 9153kg 305-day annual yield. The herd has excellent fertility (calving index 370 days, 30% conceiving of served, conception rate 44%), udder health control and overall performance. Martin would be described as an early adopter and also an advocate of SDCT. He is an Arla farmer and the SDCT extension day was delivered at his farm. Chris Gerard, herdsman is also an exceptional individual who works to a high standard. Martin and Chris always attend

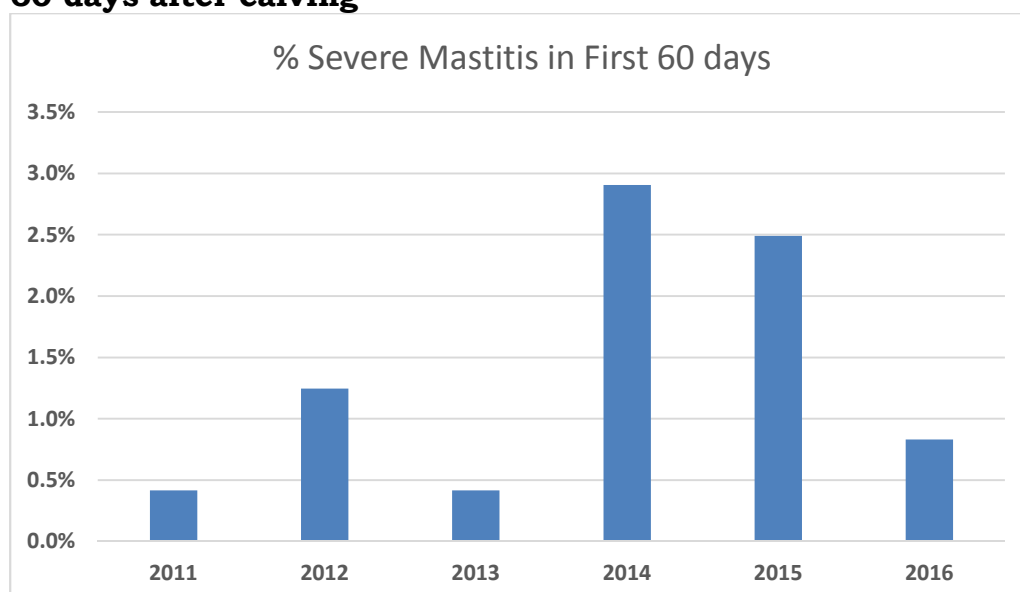
meetings and are constantly learning new approaches. All data is recorded via Interherd on the farm and shared with the practice. In August 2014 the farm changed to using recycled manure green bedding in the cubicles) and fully adopted SDCT in April 2015. A protocol developed by Andy Biggs was shared with Chris and we developed a protocol of developing a monthly report though Interherd + to segregate cows into high and low risk groups as they approached drying off. Chris also accessed data through iReports (NMR) and Interherd.

Figure 1. Incidence of mastitis at Shorn Hill Farm

Mastitis Type	Overall	2012	2013	2014	2015	2016
Overall	31%	43%	27%	33%	33%	18%
Mild	21%	32%	20%	23%	20%	12%
Moderate	3.9%	4.1%	2.8%	4.4%	5%	3.2%
Severe	5.4%	5.8%	4.0%	6.7%	7.8%	2.9%

Overall mastitis was historically well controlled on the farm (circa 30% incidence) although there were persistent challenges with more severe mastitis (5-7%). The severe cases were successfully treated in most instances and the overall culling for mastitis and SCC remained low at less than 4 cows per year. Further investigation revealed some fundamental problems with the parlour in 2015 which were corrected.

Figure 2. Analysis of the annual incidence of severe mastitis in the first 60 days after calving

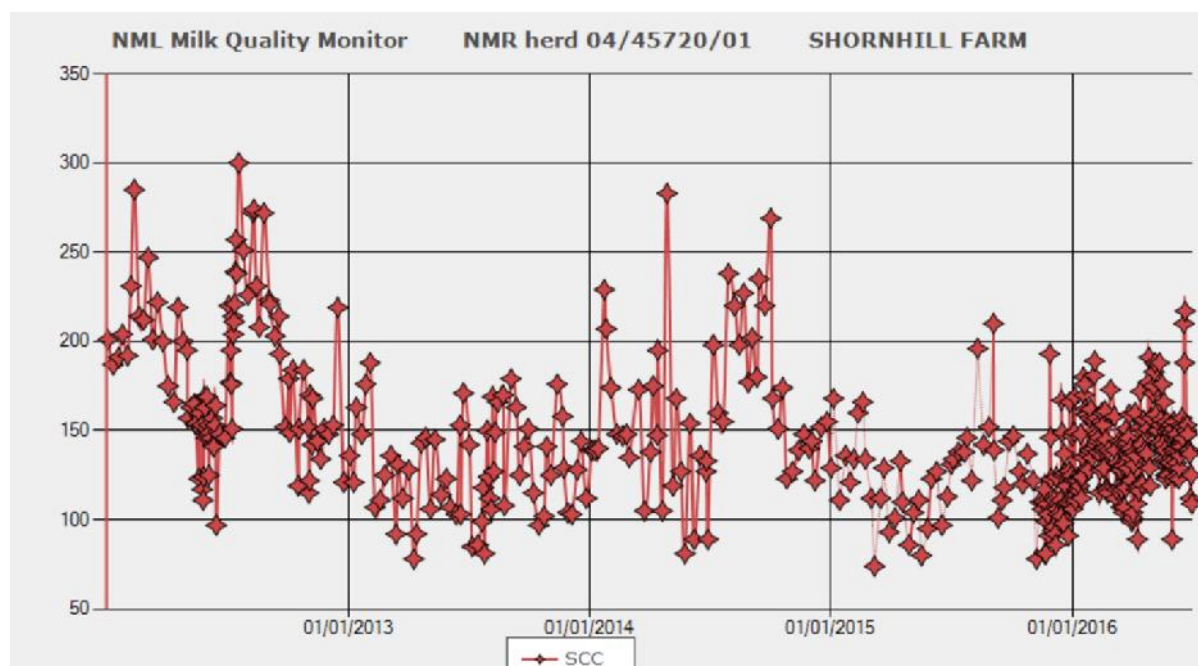


	2011	2012	2013	2014	2015	2016
No. Severe mastitis	1	3	1	7	6	2
No. Calvings	241	251	236	269	290	315
% Incidence	0.4%	1.2%	0.4%	2.9%	2.5%	0.8%

More detailed analysis of data however has shown that there has been a 3-fold reduction in severe mastitis in cows in the first 60 days in the year following adoption of SDCT and correction of the parlour faults. (Fig 2)

The somatic cell count results have remained on target and have reduced in 2016 compared to preceding years (Fig 3)

Figure 3. Bulk Somatic Cell Count Results Shorn Hill Farm



Whilst it is never possible in a temporal observational study to ascertain the significance of all the changes on the farm, Martin and Chris are confident that SDCT is the best way to dry off cows.

In September 2016 the author produced a short video for use within the practice to help other farmers to show SDCT is practised at Shorn Hill Farm. (<http://www.parkvetgroup.com/farm/dry-cow-therapy-video/>)

Farmer Attitudes and Beliefs

The farmer attitudes and beliefs regarding SDCT are critical. If the farmer does not believe in the concept, then the process required to perform the

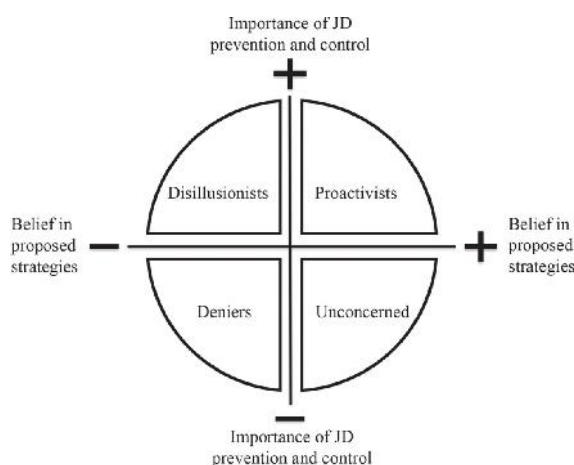
procedure will not be universally applied. Prior work has been done on farmer attitudes to mastitis control (Lam 2013). Further work focusing on the use of SDCT (Scherpenzeel et al 2016) revealed that 53% of Dutch farmers were having problems with potential negative consequences of SDCT. The top 3 concerns related to potential poor recovery without antibiotics, higher risk of sick cows and cow welfare concerns.

Jones(2015) in a UK study on farmers attitudes to antibiotic usage established that 70% of farmers thought that reducing antibiotic usage would be a good thing and the key driver to change was respondents' belief that their social and advisory network would approve of them reducing antibiotic use. Their vet's attitude was instrumental in shaping this belief.

Arla hosted a major initiative in 2015 in the UK where 40% of its producers (1750 farmers) were trained in the use of SDCT. Parallel training of 300 vets was delivered to ensure the same message was delivered. Kite Consulting ran the workshops and gathering opinions of 984 farmers to SDCT as part of the Arla SDCT initiative. At the onset of training data gathered showed that 55% of farmers were not sure that SDCT could control mastitis. 8% were convinced that SDCT would not work. Only 27% prior to commencing training believed that SDCT would work. This project revealed that 23% of farmers using SDCT had been trained by their vet, 37% self-taught and 33% by someone else on the farm. Each farmer was then trained using group work and practical workshops using artificial udders on farm. As a result of the 3-hour training session 97% of farmers were confident and able to practise SDCT. Subsequently Arla SDCT secured the World Dairy Innovation Award for work on improving sustainability and reducing antibiotic use.

Further work by Ritter (2016) investigating the farmer attitudes to Johne's disease control showed that farmers could be separated into four groups: Proactivists, Unconcerned, Disillusionists and Deniers based on analysis of the relative importance and belief they had with Johne's control. (Fig 4).

Figure 4. Graphic illustrating how farmers can be sub grouped according to their relative beliefs and importance of a disease control strategy (Ritter 2016).



This work clearly showed that each group required a different message to help them engage. Proactivists required information and engagement with control schemes whereas the Unconcerned (believed in JD control but lower priority) required information that would increase the importance of JD control such as economic impacts. The Disillusionists required more evidence that the program will work and benefit them. This is a simple methodology that can be applied to the SDCT strategy

UK Farmer Survey

A short non-random survey was conducted in September 2016 using Survey Monkey to elucidate farmer attitudes and beliefs towards SDCT. The selection criteria will be biased as respondents were sought from the more proactive groups of farmers (Tesco farmers, Herdwise users, any farmer using Twitter or working with proactive vets contacted to distribute to their farmers). The purpose of the project was to establish a clearer understanding of the farmers' fears, blocks to engagement and beliefs relating to SDCT. The aim was also to evaluate how these varied according to the farmer attitude e.g. Proactive vs Unconcerned. At the time of the production the data collection is incomplete and the full results will be delivered at a later date. 291 farmers have taken part in the survey to date.

SURVEY RESULTS

Proportion using SDCT

Two thirds of respondents were using SDCT. Full use of SDCT all year round was practised by 38%. (Fig5). 98.6% of respondents were using DCT.

Figure 5. Dry Cow approaches adopted by 284 Farmers in the UK

Dry cow strategy	% (and No.)
Selective dry cow therapy used on all cows, all year round	38.38% (109)
Selective dry cow therapy approaches 50-75% of the herd	13.03% (37)
Selective dry cow therapy approaches on less than 50% of the herd	15.85% (45)
Antibiotic Dry Cow Therapy only	7.39% (21)
Antibiotic Dry Cow Therapy and teat sealants on all cows	23.94% (68)
DO NOT USE any dry cow therapy at all	1.41% (4)
Total	284

Drivers for SDCT

80% of farmers responding believed that reducing antibiotic usage would be good for their livestock and 75% believed it would be good for people. Only 12% believed it would reduce severe mastitis and 9% believed it would lower cell counts. The importance of SDCT is clear to the majority of farmers.

Fears and Concerns

The farmers expressed significant fears and concerns about the use of SDCT with 54% of farmers fearing severe mastitis and death with no antibiotic cover. Significant concerns existed regarding cell counts not being controlled effectively and high cell counts developing (79%) and risk of more mastitis in following lactation (29%) or severe mastitis occurring (24%) (Fig 6). Whilst importance for SDCT exists the belief that this can be done safely is not sufficiently established.

Figure 6. Fears and Concerns of the application of SDCT

Fears and Concerns	% and No.
Fear of severe mastitis / cow deaths with no antibiotic cover	53.82% 141
–	44.27%
Cell counts may not be controlled as effectively	116
–	14.50%
Any change will bring risks. I am happy with blanket antibiotic dry cow therapy	38
–	28.63%
More mastitis in the next lactation	75
–	24.43%
Higher risk of severe mastitis	64
–	35.11%
Higher cell counts may occur	92
Total Respondents: 262	

Problems experienced using SDCT

Approximately half the respondents answered the question “Have you ever experienced any problems with SDCT”. The overall proportion of farmers experiencing severe mastitis (17%) and mortality after infusion (17%) would support the fears and concerns farmers have regarding SDCT. Selection bias

may have increased this figure with farmers with problems more likely to possibly respond to the survey. This figure relates to an unlimited time period. It is interesting to note that a third of the farmers that had suffered severe mastitis and death but had progressed and become proactive farmers who were willing to recommend the procedure to another farmer. A number of farmers commented that they had experienced problems in the early days of use but had now learned or had been trained to perform SDCT more effectively.

An increase in cell counts was reported by 15% of farmers (Fig 7).

Fig 7 Clinical problems experienced as a result of SDCT

	Overall	Proactivist
Severe mastitis	16.8%	6.9%
–	49	20
Deaths after infusion of teat sealant	16.8%	7.2%
–	49	21
Increase in mastitis	10.7%	1.7%
–	31	5
Increase in cell counts	14.8%	6.5%
–	43	19
Total Respondents: 138		

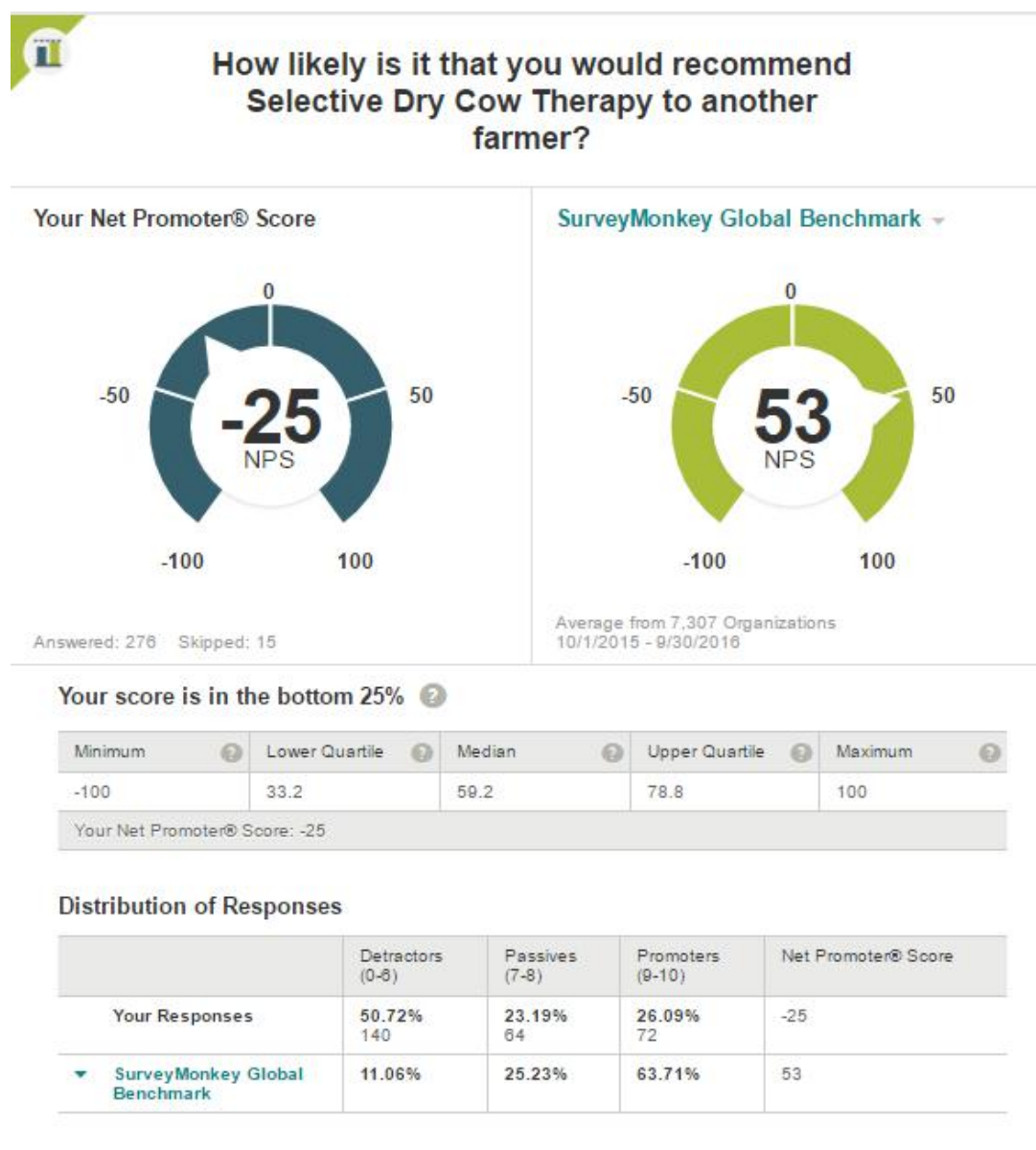
How the farmers feel about SDCT?

Approximately 50% of the farmers would recommend SDCT to other farmers (Proactivists), 18% agreed it was a good idea but low on their priority lists (Unconcerned) and 15% did not believe it worked or had bad experiences before (Disillusionists). Only 1.5% did not believe in SDCT.

The Net Promoter Score revealed a much lower level of commitment to SDCT. This has become an established methodology to assess how a customer would respond if asked whether they would recommend a company/ service or product to a friend. A successful business would target to have a large majority of customers scoring 8-10 (true promoters).

The Survey Monkey benchmark score from over 7,307 organisations for the Net Promoter is 53 and with SDCT the score is -25. 51% of respondents are classed as detractors, 23% passives and only 26% true promoters (delivering a score greater than 8 on a scale of 1-10). (Fig 8).

Figure 8. Net promoter Score values for SDCT



What would make the farmers more likely to use SDCT?

The respondents were divided into 3 groups. Proactivists (firm believers), Unconcerned (SDCT low priority or only practising SDCT as result of processor wishes) and Disillusionists (bad experiences/not interested) and analysed for the key things that the farmer would value. The farmer could only select one answer.

The Proactivists were driven by more training and help from their vet, the Unconcerned and Disillusionists wanted more convincing that SDCT works and that reward outweighed the risks. (see Fig 9).

Figure 9. Table illustrating the key elements that would encourage farmers to use SDCT more according to their belief and importance group.

	Overall	Proactivists	Unconcerned	Disillusionists
More on farm training on the technique of DCT without causing infections?	19%	38%	9%	10%
	40	27	7	3
More help from my vet on choosing which cows to treat with what?	12.00%	24%	4.00%	6.00%
	26	17	3	2
More convincing that the rewards outweigh the risks	26%	6%	37%	39%
	55	4	30	12
More evidence that Selective Dry Cow Therapy works	34%	23%	42%	45%
	73	16	34	14
Adopt milk recording so I can make decisions on which cows to treat with antibiotics	8%	10%	9%	0%
	16	7	7	0
Total	210	71	81	31

DISCUSSION

SDCT provides a huge opportunity for the dairy industry to reduce overall antibiotic usage. The journey has just begun and in order for the SDCT program to be successful the need for the program has to be clearly explained to the dairy farmers for engagement to be secured. Once this “need is secured” then a solution needs to be delivered. If we are going to capture the attention of the Unconcerned or Disillusionist farmer groups, then simply providing training events may not be enough. They may not attend. There are a huge number of competing priorities for a dairy farmer to achieve and without greater clarity and coherent messages from all parts of the industry SDCT will not be universally and successfully deployed. The Arla SDCT project achieved 40% attendance which is a huge achievement. How do we access the remaining 60%? How do we target the farmers who have not had the benefit of such intensive training?

The anecdotal reports of mastitis and mortality after infusion of teat sealant alone is supported by the findings in this survey. These are seldom reported by the farmers as they appreciate that this is not a product failure per se. Teat sealants applied in a hygienic manner by well-trained farmers, who are committed to high standards, will deliver results. The opposite equally applies and these adverse events will dissuade others from making the effort to progress to adopting the rigours of SDCT. BDCT is much easier and simpler to apply and has been prior to SDCT universally supported by 99% of farmers in the UK. (Berry 2002). The importance of the herd risk factors, the balance of pathogen load within a herd (which will change with the application of SDCT) needs to be more carefully explained to the veterinarians. SDCT has risks and these have to be carefully managed and the overall surveillance for mastitis pathogens needs to be increased in herds practising SDCT.

However, for the SDCT program to be a success there are a significant number of challenges to surmount:

- How can significant mortality be prevented with the use of internal teat sealants alone in cows? How can you get the disillusioned farmers to attend training? What is the best way of getting the message across to all the industry?
- How will over 9000 farm businesses and all their workers involved in DCT be successfully trained in SDCT?
- How can the proactive farmers be used more effectively to promote SDCT?
- How can all farmers be more convinced that the SDCT works? All groups are still nervous about the impact of the program and the Net Promoter Score is negative with more detractors than promoters.
- Who will support and finance the program?
- Who will host and manage a program and education program of this scale?
- The program is unlikely to be adopted voluntarily. How can farmers be incentivised to do more to reduce antibiotic usage?
- What resources can be provided more effectively from a co-ordinating body to assist in the program?
- What further research is required to support the program?
- What role does the vet have in supporting SDCT? What are their views and recommendations? Are they up to date with most appropriate advice on SDCT technique and selection criteria (both individual and herd risk factors)

CONCLUSIONS

Great opportunities and challenges exist in terms of more widespread adoption of SDCT. With careful planning and co-ordination of inputs SDCT could be successfully applied with effective results. However, there are a

number of hurdles that will need to be overcome if the greater majority of dairy producers are to engage with the program in the UK.

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Useful web links

- Selective Dry Cow Video www.parkvetgroup.com/farm/dry-cow-therapy-video/
- Infusion of dry cow and lactating cow products-AHDB dairy

- Best practice Administration of Teatseal/Orbseal and Dry cow therapy
<https://www.youtube.com/watch?v=HeKfL8BRGss>

ACKNOWLEDGEMENTS

Thanks to the farmers and vets that encouraged the distribution of the questionnaire, Martin Beaumont and Chris Gerard for their patience and colleagues from the Park Vet Group for their support. Particular thanks to Andrew Biggs for guidance on current techniques of SDCT.

NOTES

COST-EFFECTIVENESS OF ON-FARM CULTURE FOR THE TREATMENT OF CLINICAL MASTITIS

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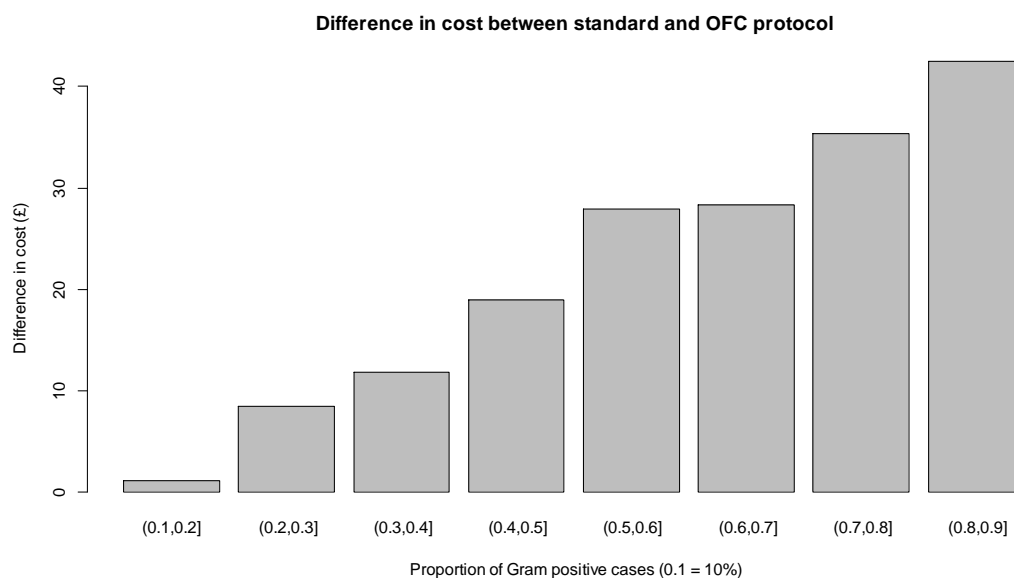
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Not only is mastitis important in terms of the economics, but the treatment and prevention of mastitis is widely reported as the most common reason for antimicrobial drug use on dairy farms ¹⁻³. Conventionally, all cases of clinical mastitis would receive a course of antimicrobial agents but an alternative approach is the selective treatment of cases according to the results of an on-farm culture (OFC) system. With the OFC system, only cases that yield a Gram-positive or mixed-culture are treated with antimicrobial drugs resulting in many cases of clinical mastitis not being treated at all ⁴. The purpose of this research was to use probabilistic sensitivity analysis (PSA) to investigate the main factors that influence the cost-effectiveness of an OFC approach to treating clinical mastitis. A specific aim was to identify the herd circumstances under which an OFC approach would be most likely to be cost-effective.

A stochastic Monte Carlo model was developed to simulate 5000 cases of clinical mastitis at the cow level and to calculate the associated costs simultaneously when treated according to 2 different treatment protocols; i) a standard approach (3 tubes of intramammary antibiotic) and ii) an OFC programme, whereby cows are treated according to the results of OFC. Model parameters were taken from recent peer reviewed literature on the use of OFC prior to treatment of clinical mastitis. Spearman rank correlation coefficients were used to evaluate the relationships between model input values and the estimated difference in cost between the standard and OFC treatment protocols.

Across all 5000 simulated cases, the standard protocol was the most cost-effective 68% of the time. The median cost related to a case treated with the standard protocol was £365 and the median cost related to a case treated with the OFC protocol was £382. The maximum difference in cost between the two protocols was £226 with a median of £19. The difference in cost between the two protocols was most closely related to the difference in bacteriological cure rate and the proportion of Gram-positive cases. As the difference in bacteriological cure rate and proportion of Gram-positive cases increased, the difference in overall cost became higher, making the OFC protocol less cost-effective than the standard protocol (Figure 1).

Figure 1. Difference in cost between standard and OFC protocol (all scenarios) taken from a model designed to simulate the cost of a case of clinical mastitis treated according to different treatment protocols A positive value for difference = standard protocol more cost-effective.



The OFC approach appears to be suitable for herds in which Gram-negative pathogens are responsible for most clinical mastitis and where the treatment of cows according to the results of an OFC approach results in minimal reductions in the bacteriological cure rates. The results highlight an ethical dilemma surrounding reduced use of antimicrobials for clinical mastitis since it could result in financial losses and poorer cow welfare in many instances.

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NOTES

IDENTIFICATION OF POTENTIAL MARKERS IN BLOOD AS A RISK FACTOR FOR OCCURRENCE OF BACTERIOLOGICALLY POSITIVE QUARTER MILK SAMPLES IN DAIRY COWS DURING THE TRANSITION PERIOD

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The transition period from late gestation to early lactation is characterized by sudden changes in metabolic and immune functions (Contreras and Sordillo, 2011), which play a critical role in the maintenance the cow's health. Thus, it is not by chance that during this period, dairy cows experienced most infectious diseases and metabolic disorders. Among the infectious diseases, mastitis is the most common and costly disease of dairy cattle.

The present study sought to identify potential blood markers that were associated with the occurrence of mastitis in dairy cows under different dry therapy strategies during the transition period. Here, we used 34 Holstein cows that were aleatory divided into three groups: cows that received antimicrobial (CEPRAVIN[®], MSD, Brazil) treatment in all quarters at drying-off (n = 10 cows, 40 quarters), cows that received internal teat sealant (TEAT SEAL[®], Zoetis, Brazil) in all quarters at drying-off (n = 11 cows, 44 quarters), and control untreated dairy cows (n = 13 cows, 52 quarters). We collected quarter milk samples and blood samples at drying-off (60 days before the expected day of parturition; M1), at parturition (M2), and three (M3), seven (M4), 15 (M5), 21 (M6) and 30 (M7) days after calving. Blood samples were collected for determination of the leukogram (total leukocytes count, and the percentage and the absolute values of neutrophils, eosinophils, basophils, monocytes and lymphocytes), hemogram (total erythrocyte count, hemoglobin concentration, hematocrit, platelet count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and mean corpuscular volume), and the serum concentration of calcium, phosphorus, total protein, albumin, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, beta-hydroxybutyrate (BHBA), non esterified fatty acids (NEFA), urea and creatinine, and the activities of alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase, and the plasma concentration of fibrinogen.

The variables under examination were first analyzed individually to verify the significance, and then in combination to assess the effect of the single variables on all others. In the first stage of the analysis, unconditional logistical model for each variable related to the bacteriologically positive quarter milk samples P -values ≤ 0.20 were considered as a select variable passed to the next stage of the analysis. In the final model variables selected in the first stage were used to develop a final model in a multivariate logistical model with $P \leq 0.05$ considered to be retained in in the final model.

A logistic regression model was adjusted, taking the following as explanatory variables ($P \leq 0.20$): treatment, moments, hematocrit, mean corpuscular hemoglobin concentration, serum total protein, albumin, urea, BHBA, cholesterol, HDL, LDL, triglycerides and phosphorous concentrations. In this model, it was found that only some variables were statistically significant ($P \leq 0.05$) for estimating the likelihood of having bacteriologically positive quarter milk samples, and these remained in the final model, as following: antimicrobial treatment (odds ratio = 0,371, $P = 0.01$), and total protein (odds ratio = 1,632, $P = 0.04$) and triglycerides (odds ratio = 1.021, $P = 0.02$) contents.

Although, some widely studied variables related to metabolic disorders during the transition period, such as BHBA, NEFA and glucose (Rezemand et al., 2007; Moyes et al., 2009), were not eligible to enter in the logistic regression model to identify which blood markers are related to high milk SCCs, others variables that can be easily and quickly determined under field conditions, such as the measurement of total protein, were associated with bacteriologically positive quarter milk samples in dairy cows during the transition period.

ACKNOWLEDGEMENT

The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Project n. 2012/08982-4). AMMPDL is indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for her fellowship. KRS and FNS are indebted to FAPESP to their fellowships.

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NOTES

IMPACT OF THE AHDB DAIRY MASTITIS CONTROL PLAN

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The AHDB Dairy Mastitis Control Plan (DMCP) provides a structured approach to mastitis control. Trained Plan Deliverers provide a diagnosis based on udder health records and work with farmers to identify and prioritise appropriate interventions. Plans are drawn up with the aid of software (the 'ePlan'). This research based scheme has been in operation since 2009 and the outcomes of the first three years of the project have been reported previously (1). This poster summarises the impact of Plans reported between 2013 and 2016.

Numbers of registered Plans were captured by automated data feed from the ePlan software. Where suitably authorised, Plan Deliverers submitted herd data (somatic cell count and, where available, clinical mastitis) from farms with Plans, for aggregation and anonymous analysis. Data were collated and parameters for analysis were extracted using TotalVet (QMMS Ltd/SUM-IT Computers Ltd). The performance of each herd was assessed in the year prior to implementation of the Plan, and each year thereafter for up to three years. The financial impact of implementation was estimated, assuming a cost of £300 for a chronic high cell count cow and £250 for a clinical case of mastitis. The cost of implementation was based on a consultant/vet fee of £1000 in Year 1 and £500 per year thereafter, plus £10/cow in herd/year spent on interventions.

Impact could be analysed over at least one year for 231 farms, and for 35 farms for the full three year period of analysis (Table 1).

Table 1. A summary of udder health parameters from herds implementing the AHDB Dairy Mastitis Control Plan (median values presented)

Year of Plan Implementation	0	1	2	3
n (SCC Data)	231	111	68	35
Bulk Milk SCC (,000/ml)	200	184	168	176
Lactation New Infection (%)	8.50	8.30	7.35	8.00
Fresh Calver Infection Rate (%)	19.0	18.40	17.60	17.15
% of Herd >200K	21.60	20.90	19.15	19.50
% of Herd Chronically Infected	14.00	13.70	12.50	11.75
n (Clinical Mastitis)	194	93	57	31
Incidence Rate of Clinical Mastitis (/ 100 cows/year)	44.0	37.0	44.0	35.0
Incidence Rate of Cows affected (/ 100 cows/year)	44.5	39	46	38

Over all timeframes there was an improvement in all somatic cell count parameters, whilst clinical mastitis parameters improved over the three year monitoring period. Over a three year period bulk milk SCC dropped by 24 x10³ cells/ml; a relative decrease of 12%. The proportion of the herd above 200,000

cells/ml and chronically infected fell by 9.7% and 16.1% respectively whilst the rate of clinical mastitis decreased by 20%. Based on a herd size of 213 cows (median for the farms submitted) and estimated costs of a chronically infected cow and a clinical mastitis case of £300 and £250 respectively, the average reduction in the cost of mastitis on each unit was calculated as outlined in Table 2.

Table 2. A summary of the financial benefits of implementation (per farm)

	Year 1	Year 2	Year 3	Cumu- lative Year 3	Estimated cost saving
No. of chronic cows saved	5	23	34	62	£18,485
No. of clinical mastitis cases saved	34	0	44	77	£19,364
Total saving					£37,849
Estimated additional cost of implementation*					£8,390
Estimated net benefit of Plan implementation					£29,459

*based on a consultant/vet fee of £1,000 in year 1 and £500 thereafter, plus £10/cow in herd/year implementation costs.

The estimated net benefit per farm, of Plan implementation, was £29,459 (£138.31/cow) over the three year period or £46.10/cow/year. Based on the number of Plans (1,044) put in place and the number of cows known to be on Plan farms (via data feeds from the ePlan software) the estimated total net financial benefit of the Plan to farmers was between £11 and £12 million per year.

Over a three year period, implementation of the DMCP has resulted in a reduction of 20% in the rate of clinical mastitis and an improvement of 10 – 16% in udder health based on SCC parameters. The estimated benefits of implementing the DMCP on farm have been shown to be in the region of £40 per cow in herd per year, after costs of implementation have been deducted. The scheme provides a structure for ongoing contact and communication between veterinary advisers and farmers, and sustained benefits in terms of udder health.

ACKNOWLEDGEMENTS

The work was funded by AHDB Dairy as part of the AHDB Dairy Research Partnership. We are grateful to the farmers, vets and consultants who submitted data.

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NOTES

'IT'S THE DOLLAR VALUE... ISN'T IT?' FORM, FUNCTION AND EFFICACY OF VETERINARY ADVICE FOR FARMER BEHAVIOUR CHANGE: A QUALITATIVE INVESTIGATION

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BACKGROUND

In the UK dairy industry, mastitis is an endemic disease that represents both a financial and emotional burden for dairy farmers (1) and a less than ideal state for the dairy cow (2). Despite significant advances in scientific research into the risk factors and management strategies implicit in its occurrence, mastitis remains a significant issue for many farms (3), suggesting implementation of this research in management and on-farm housing is still inadequate.

Farmers increasingly rely on veterinarians as their primary source of information for mastitis management, meaning their engagement and compliance with veterinary advice is critical to generate meaningful improvements in incidence rates (4). This places veterinary communication at the heart of the issue of mastitis reduction. However, whilst veterinarians recognise their influence and the need to be proactive communicators, they struggle with acting upon this awareness in daily practice (5). Communication of this advice is complex, with veterinarians needing to simultaneously convey accurate and up-to-date expertise on management whilst promoting farmer engagement with behaviour change (whether administering treatments, enacting management processes or a multitude of other actions).

In order to enhance the uptake of veterinary advice, a deeper understanding of cattle veterinarian communication and its effects on client motivation is essential. This study offers unique qualitative insight into how cattle veterinarians currently communicate on matters of herd health (mastitis and lameness), allowing reflection on the possible motivation of veterinarians within them, and the resulting effect on client participation. Recommendations to enhance the uptake of advice are made in conclusion.

MATERIALS AND METHODS

Role-play sessions reflecting consultations on mastitis and lameness were recorded between cattle veterinarians (n=15) recruited from UK practices located in South West England and an actress experienced in role-play scenarios in both medical and veterinary education. The actress was provided with a character and farm profile reflecting a 'typical' UK situation, indicating mean herd size, productivity, lameness and mastitis levels. Background information on the farmer's family, perceived barriers to uptake of advice and attitudes/norms/perceived control of lameness and mastitis were provided. During each 'consultation', veterinarians were provided with a short excerpt of the disease issue on the farm, an indication of the risk factors that were likely

to be involved, and evidence to encourage them to broach a broad topic area of change with the farmer. For lameness, the broad topic was early detection and treatment of lame cows; for mastitis, it was use of the AHDB Dairy Mastitis Control Plan. Role-play interactions were transcribed (*verbatim*) and analysed thematically (6) using the qualitative software NVivo 10 (QSR International). The entire dataset was coded using inductive themes (i.e. themes determined by the dataset and not *a priori*).

RESULTS

Consultations lasted an average of 11.2 minutes (range 7.7 to 14.9). Thematic analysis revealed three prominent themes: firstly, the *language of the advisory process*, encompassing the effects of verbal framing of both disease and control mechanisms; secondly, the *consultation strategy*, where typical veterinarian approaches to shaping advisory discourse emerged; thirdly, *building the interpersonal relationship*, reflecting interactions underpinning how the veterinarian-farmer relationship was established.

CONCLUSIONS

The value of veterinary advice on mastitis rests not only on the recommendations themselves, but on engaging farmers in the behaviour change necessary to ensure these recommendations are effective. Consideration of these results (full details provided upon request) could offer those working in advisory contexts the opportunity to enhance their delivery of mastitis recommendations, thus increasing the potential for successful uptake of advice.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support and funding provided by the British Veterinary Association Animal Welfare Foundation, without which this work could not have been completed. We would also like extend our gratitude to our participating veterinary practices who enabled us to achieve our research goals and Judi Laister whose acting skills brought our role play scenarios to life.

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NOTES

LATEST DEVELOPMENTS IN AUTOMATED PARLOUR TECHNOLOGY TO DETECT MASTITIS

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SUMMARY

Mastitis is one of the main production diseases of dairy cows. Presence of high levels of mastitis pathogens, in raw milk at least, can constitute a risk to human health and is regarded as an indication of less than adequate milking methods.

There are also many published scientific papers discussing a myriad of possibilities for automated sensors for mastitis. In spite of this, the range of technologies being used in practice is relatively limited. This paper sets out to discuss the application of sensor technology as a tool for compliance with legislation and to manage udder health.

MASTITIS - THE DISEASE AND SYMPTOMS

Mastitis is not one disease but can be caused by a large number of pathogens.

Dairy Hygiene Legislation requires that “that milk from each animal is checked for organoleptic (taste, colour, feel, smell) or physico-chemical abnormalities by the milker or a method achieving similar results and that milk presenting such abnormalities is not used for human consumption” (15). With automated milking systems where a human milker is either not present, or does not have time for observation, sensors are available to replace or assist operators and can be used as a basis to divert abnormal milk. The “gold standard” is that which can be achieved by a human.

Clinical mastitis signs vary from seeing a few clots in the milk with perhaps some swelling of the infected quarter to severe signs which include swollen quarter or whole udder. The cow may have a high temperature, fever, loss of appetite, and be dehydrated (3). In severe cases, the disease can result in the death of the animal.

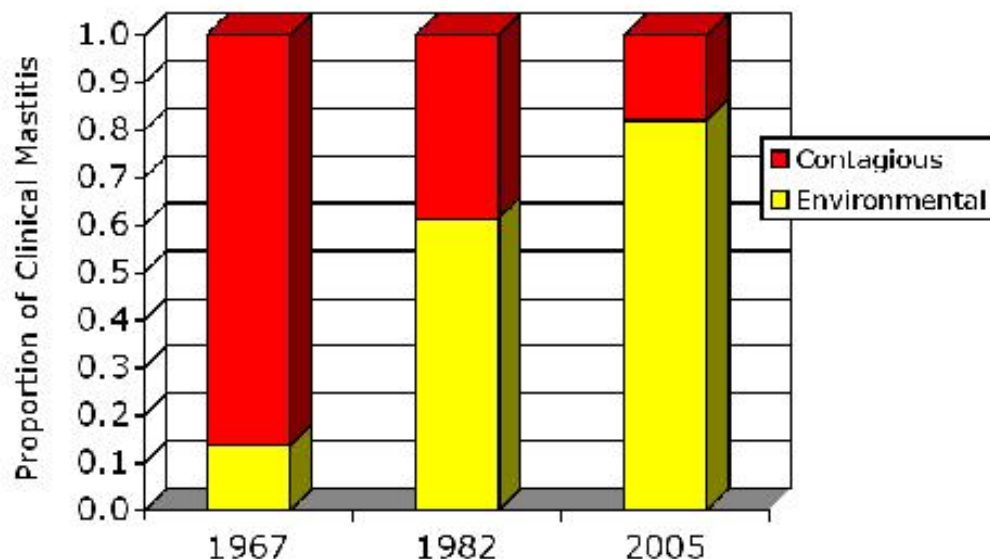
Sub clinical mastitis is when there is bacterial infection present with no clinical signs. The cow could have a raised somatic cell count (number of white blood cells/ml of milk) and there may be bacteria in milk cultures. However, legislation does not demand that such milk is rejected.

It is generally accepted in the UK that a somatic cell count of >200,000/ml of milk is an indication that a cow or quarter is infected. Care has to be taken when using this figure as a cow could have a cell count just below 200,000 and have $\frac{3}{4}$ with a cell count of less than 50,000 and $\frac{1}{4}$ with a cell count well over 200,000/ml. Where a herd risks being penalised on the basis of somatic cell counts, rejection of milk from cows known to have a high somatic cell count can be a useful milk quality management tool. The cost of that milk must be weighed against the likely penalty.

It is difficult to find up to date statistics on mastitis incidence. Relatively recent survey results indicate that annual incidence remains, stubbornly, at an average of 43-71 clinical cases per 100 cows per year (3)(1).

One significant trend is the change in the relative proportions of contagious and environmental mastitis over the last 40 years. Successful implementation of mastitis control strategies has reduced contagious infection level to less than 20% of the total (3). The wide range of degree of severity and symptoms gives a hint to the difficulty in applying sensor technology.

Table 1. Contagious and Environmental Clinical Infections 1967 – 2005 (3)



SENSOR TECHNOLOGY FOR DETECTING MASTITIS

There are a large number of patents relating to the use of mastitis sensors on automatic milking units. In the wider context, a patent search on “mastitis sensor” indicates that there are at least 1461 (6) patents applied for or granted. It is not practical to list all of the technologies which have

been the subject of these applications. Some of the technologies proposed as a basis for possible sensors include but are not limited to:

- Milk lactate (4)
- Milk lactose level (9)
- Acute Phase Proteins MAA (5)
- NAGase (13)
- Electrical Conductivity of Milk (8,12)
- Colour/Blood (16,17)
- Somatic Cell Count (16)
- Viscosity, California Mastitis Test (18)
- Thermography (11)
- Adenosine TriPhosphate ATP as an indicator of cells (7)

Although not specific technologies, the following can also be used as additional supporting indicators of mastitis infection:

- Drop in milk yield
- Not attending milking robot, or reduced attendance
- Changing behaviour

When considering the application, relevance and relative merits of sensor technology in respect of mastitis detection, it is useful to refer to the mastitis cause-effect spectrum explained by Claycomb et al. (2). It is also useful to take into account the differing symptoms associated with various types of infection. The decisions based on sensor output may vary depending on whether the main driver is milk quality, or mastitis treatment.

In the context of automatic milking, the last thing an operator wants or needs is to be burdened by mastitis alerts where there are no visible symptoms, where intervention may not to be justified or has a negative cost benefit. However, there is a contrasting argument, from the point of view of udder health management, that early warning of impending problems can be improve treatment success rates. This highlights the fact that it is essential to educate users in respect of the correct use of sensor technologies.

SENSORS IN PRACTICAL USE FOR MASTITIS DETECTION

In 2004, in a paper with a similar brief, Reinemann (16) reported on the basis of European and American studies that electrical conductivity and milk colour were the most widely used on-line milk sensing methods. He also noted that, deviation in milk yield and milking interval are widely used supporting diagnostic techniques. He also predicted that cow side measurements of somatic cell count were likely to be applied to automatic milking in the future.

Whilst, superficially, the situation would seem to be largely similar today, a number of significant observations can be made.

Most sensor technology implemented today has been driven by the need to detect, or at least predict, onset of clinical mastitis symptoms.

An ideal sensor should be non-invasive, cleanable, avoid damage to the milk and not require the use of reagents, particularly where those reagents may be dangerous to the environment or difficult to dispose of. This point has ensured that sensor technologies such as electrical conductivity and optical sensing methods, including colour, continue to be attractive. The use of such sensors also strikes a balance between effectiveness and low running cost.

Although the use of electrical conductivity has been discussed, supported and condemned for many years, the main point to note is that there have been significant developments in the refinement of its use.

Significantly, Hamman & Zecconi (8) concluded that “within cow comparison between quarters seems to be the best way to use conductivity measurements” and “measurement of electrical conductivity in milk requires a high degree of standardisation”. These conclusions indicate that the main challenge with electrical conductivity measurement is in standardisation of the measurement technique and interpretation of the physical data.

Mein et al (12), using temperature correction and more sophisticated algorithms to take account of milking interval and other factors, have shown a sensitivity of 92% and specificity of 95% where a true positive was defined

as the presence of a major pathogen. A further study was carried out by the same authors on 4 herds and reported sensitivity of 80% and specificity of 93%. In that study a true positive was defined as a cow with visible clots in the foremilk of one or more quarters at a monthly herd visit, reported in the same paper reported. True negatives had no visible signs of clots in their milk and low CMT (scores of 1 or 2). The sensitivity and specificity data achieved are favourably comparable with that reported for human observation by Hillerton (10).

Comparison of conductivity from one quarter (or, in the case of goats, one half) with that of milk from other teats is a basis for much higher success rates. This technique is used, with high success rates, in robotic milkers and in goat milking systems.

The predicted (16) use of cow-side somatic cell counting or estimating is not regarded as an indicator of abnormal milk (14) and has not, by any means, been universally adopted.

The use of optical technology to measure various milk components, including blood, is now in common use, whilst still satisfying the ideal of being non-invasive and not requiring reagents.

The final comment from Reinemann (16), that inputs from a number of sensors, including milk composition, animal behaviours and milking characteristics need to be co-ordinated via a “smart” system, is as true now as it was then. This is particularly relevant when considering the predominance of environmental pathogen related mastitis. Often the first symptoms can be failure to attend the automatic milking machine and/or loss of milk production. For many farmers, one of the most important sensors is the list of cows not attending the machine.

Research has demonstrated the potential for use of thermographic camera technology (11) to identify infected areas of udder tissue, as “hotspots”. Until now, the cost of such technology has been prohibitive. However, the availability of low cost devices is likely to create interest in its use.

There are a variety of derivatives of laboratory techniques for measurement of Somatic Cells, ATP, Lactate forming the basis for line side measurement.

CONCLUSION

An enormous array of technologies are theoretically possible for detecting mastitis related characteristics within milk.

The application of such sensing technology is driven by legal requirements to detect and divert abnormal milk.

New technologies must provide a very clear benefit at a cost which is recoverable.

Sensors should, ideally, be non-invasive, not damage milk, nor require discard of milk and not require reagents.

The most important factor in sensor technology for mastitis is an intelligent management system to combine the output of various sensors and permit “management by exception”.

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SUMMARY OF PAPERS PRESENTED AT THE 6TH IDF MASTITIS CONFERENCE 2016

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TREATMENT OF MASTITIS - IS THERE ANYTHING NEW?

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Antimicrobial treatment of mastitis has remained more or less unchanged for decades. The most common approach is intramammary administration of broad-spectrum antibiotics or combination of antibiotics. Use and drug selection for systemic administration of antimicrobials is often haphazard and not supported by efficacy data. Milk samples are seldom taken systematically for microbiological analysis. In dairy herds treatment protocols designed by competent veterinarians are rare. Some alternatives to antimicrobial substances have been tested in laboratory conditions but very few have any real promise. Their development to authorized products is far in the future if ever.

Prudent use guidelines for the treatment of mastitis have been established in some countries, but their extent and degree of details vary. The basic principle in all antimicrobial use should be targeting treatment towards the causing agent. In clinical situations treatment of mastitis is initiated before microbiological diagnosis, but an aseptic milk sample should always be taken for bacteriology. New PCR-based methods have hastened diagnostic procedures. Media which allow rapid (over-night) diagnosis are available to be used in clinical mastitis. In addition to support treatment decisions, bacteriological data provide valuable information of the distribution of mastitis causing agents in the herd, which can be used to target preventive measures. In large farms at least in USA and Canada simple on-farm diagnostics has become into use and has guided treatment decisions, reduced costs and helped in residue avoidance.

The Nordic approach is to target treatment towards the most common pathogens in the herd. Benzylpenicillin as the first choice for treatment of mastitis due to streptococci and penicillin-susceptible staphylococci. Unfortunately, antimicrobial products which would be suitable to targeted treatment are not available in all countries. Instead, old products authorized decades ago with very limited clinical documentation are still on the market. Third or 4th generation cephalosporins are commonly used as standard treatments in the EU and USA. They belong to critically important antimicrobials and should be avoided in the treatment of food animals. Treatment duration should be economically justified but long enough to be efficient. Supportive evidence for the superiority of extended treatment exists only for mastitis caused by *S. aureus* and *Str. uberis*. Systemic treatment may improve efficacy in *S. aureus* mastitis. Evidence on the efficacy of antimicrobial treatment of coliform mastitis is weak and supportive measures are most

important. Treatment of subclinical mastitis is in general not economic and can be postponed until drying-off, with some exceptions like *Str. agalactiae* outbreaks.

In mastitis control, preventive measures are of major importance. When needed, antimicrobial treatments should be efficient, economic and comply with prudent use principles. In dry cow therapy, present trend in the EU is to move from blanket therapy to selected therapy, due to changes in the legislation based on concerns on antibiotic resistance. Conclusion is that there is very little new in mastitis treatment. However, enough scientific knowledge is already available to enable appropriate mastitis treatment.

EFFECT OF DRY COW TREATMENT STRATEGIES BASED ON QUARTER SPECIFIC DIAGNOSIS – POTENTIAL FOR REDUCTION OF ANTIBIOTIC CONSUMPTION

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Antibiotic treatment of food producing animals is under discussion due to development of antibiotic resistance in food borne pathogens. In dairy production, the treatment of cows at drying off with long lasting antibiotics without knowledge of the infection status of the udder is of major concern. A significant reduction of antibiotic consumption could be achieved by restriction of treatment at drying off to infected quarters instead of blanket dry cow treatment. The aim of the study was to investigate how a quarter-specific use of antibiotics at drying off affects the outcome of the dry period.

The study was carried out on two research stations (MRI, TI) from 2013 to 2015. Cows were sampled weekly over the last three weeks prior to drying off and three weeks after calving for cyto-bacteriological analyses. A quarter was defined as infected if in two of three samples the same pathogen was identified. Cows were split up into four experimental groups: G0 - cows without infections did not receive any antimicrobial drug. Cows with at least one infected quarter were assigned to the following treatment groups: G1 – only infected quarters were treated with antibiotics, G2 - all quarters were treated with antibiotics, G3 – quarters were not treated at all. Cows with clinical mastitis before drying off were excluded from the study. Based on resistance tests gram-positive pathogens were treated with a dry cow drug containing 1000 mg oxacillin, for cows infected with gram-negative pathogens a combination of 100 mg Framycetin, 280 mg Benethamin-Penicillin and 100 mg Penethamat was applied.

In total, 216 dry periods of 164 cows were included; for 722 (83.6%) of all sampled quarters complete data sets were available and used in statistical analyses. At MRI, 78 (20.3%) and 22 (5.7%) of 384 quarters were infected by major or minor pathogens, respectively, while at TI minor pathogens predominated (146 (43.2%) of 338 quarters versus only 4.4% infections by major pathogens). Cure rates of infected quarters treated with antibiotics were

85.7% and 70.0% in G1 and 64.2% and 60.3% in G2 at MRI and TI, respectively. Self cure rates of infected quarters without any antibiotic treatment were approx. 40% (8 of 19 (MRI) and 19 of 48 (TI)). For uninfected quarters the new infection rate without antibiotic treatment was 28.0% (113 of 403 quarters) and did not deviate significantly from uninfected quarters in cows with total dry cow treatment (31.0%, 18 of 58 quarters).

The results indicate that quarters without infection at drying off might not benefit from antibiotic treatment irrespective of the infection status of the cow's other quarters and the udder health situation on the farm as long as hygienic standards are high. These findings suggest that by selective treatment of infected quarters at drying off the use of antibiotics in dairy farming could be reduced considerably without impairment of udder health.

ANTIMICROBIAL PREVENTION OF NEW IMI IN THE DRY PERIOD REVISED

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Introduction

The clinical guideline "Use of antimicrobials at drying off" was implemented in the Netherlands in January 2014, with the aim to reduce preventive use of antimicrobials in the dairy industry. The guideline prescribed dry off without antimicrobials for primiparous animals with a SCC <150,000 cells/ml and multiparous animals with a SCC <50,000 cells/ml at the last test date before drying off. Selective DCT instead of blanket DCT caused anxiety in both farmers and veterinarians. The objective of this study was to investigate the effects of this guideline on udder health performance at herd level.

Material and Methods

Data were collected through the University Farm Animal Practice, which services around 350 dairy herds. Total sales of intra-mammary tubes were collected for 2012-2015. Defined Daily Dose Animal (DDDA) was available for 2013 and 2014 per administration route at practice level, but not yet for 2015. Additionally, udder health performance data from herds that participated in the DHI were used. The yearly mean % of new IMI and mean % cured animals in the dry period were calculated. Threshold SCC for multiparous animals was 250,000 cells/ml and for primiparous animals 150,000 cells/ml. A new IMI in the dry period was defined as a SCC below the threshold before drying off and above the threshold at the first test day after parturition. Cured animals changed from above to below the SCC threshold over the dry period. Preliminary results include changes in antimicrobial usage over 2012-2015 compared to udder health from 2013-2015.

Further analyses will include multivariable analyses with important herd characteristics such as herd size, herd age, milk production level, and AMS versus conventional milking as well as different antimicrobial classes.

Results

Sales figures over 2012-2015 show a clear decline in dry cow tubes, after some stocking in 2013, while internal teat sealers showed a steady increase (Table 1).

Table 1. Total sales of intra-mammary tubes for approximately 27,000 cows.

Year	Dry cow tubes	Mastitis tubes	Internal teat sealers
2012	43,605	34,237	10,421
2013	52,181	35,952	14,966
2014	34,614	29,412	20,668
2015	32,314	29,831	25,833

The overall mean DDDA decreased from 3.0 in 2013 to 2.2 in 2014. Antimicrobial use for DCT decreased from 1.5 to 0.9 and use for mastitis changed from 0.7 to 0.6 DDDA.

Udder health indicators showed a slight increase in new IMI and no important trend in cured IMI (Table 2).

Table 2. New IMI and cured IMI over the dry period (N = 280 dairy herds).

Year	New IMI		Cured IMI	
	Mean %	Median %	Mean %	Median %
2013	16	15	74	75
2014	18	17	74	77
2015	17	15	76	78

Conclusion

Implementation of the guideline per 2014 led to a reduction in antimicrobial usage for DCT and, unexpectedly, also for mastitis treatment. No important increase in new IMI during the dry period was seen. Results of this study may reduce anxiety around more prudent use of antimicrobials, including antimicrobial reduction at drying off.

DEVELOPMENT OF AMS IN THE NORDIC COUNTRIES, 1996-2015

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Automatic Milking Systems (AMS) were introduced into the Nordic countries in 1996. Since then there has been a dramatic change in the development of both the milking technique and the general environment for milk production in those countries. At the end of 2015 the total number of farms with AMS in all

the Nordic countries was 4.444¹ (31/12/2015), showing only a slight increase during the previous year from 4.293² (31/12/2014). The number of AMS boxes was however 7.179 (31/12/2015), showing a slight increase from 6.894 (31/12/2014).

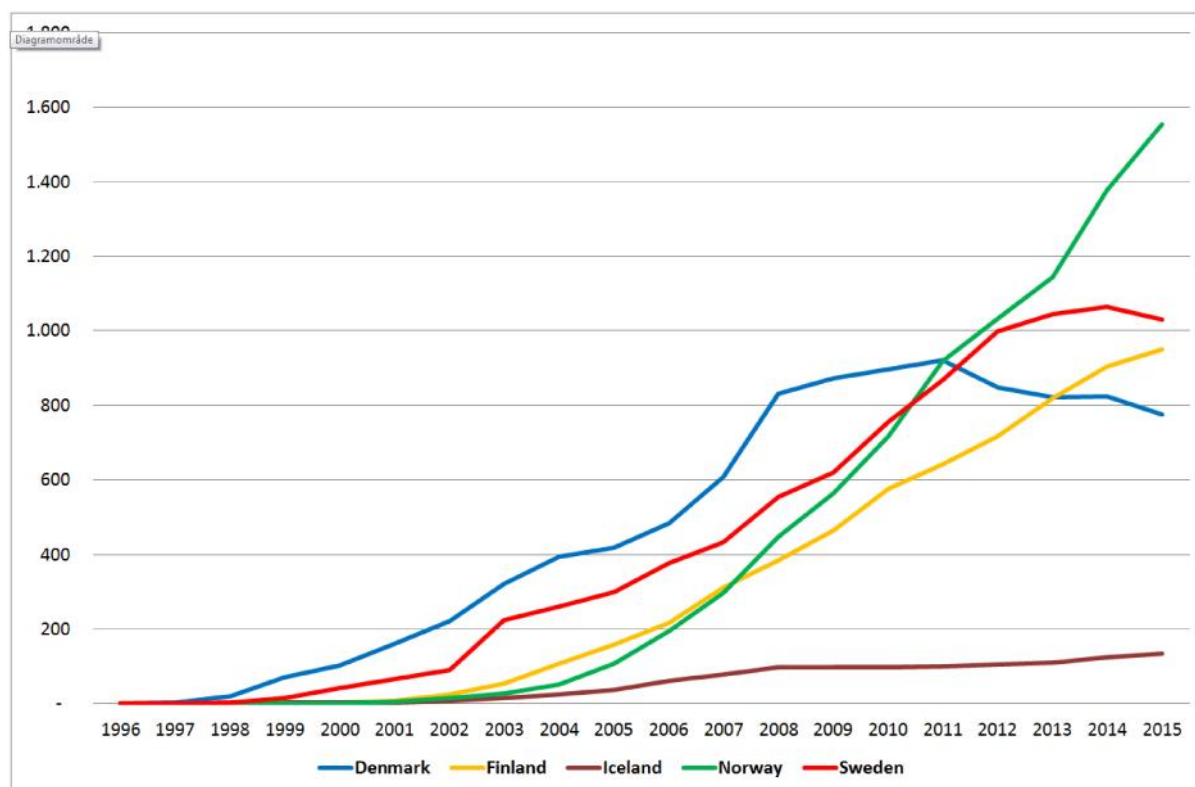
The development in recent years is quite different between the Nordic countries (Graph 1). Especially interesting is the development in Denmark where the increase in the number of farms with AMS has stopped and since 2012 the number of AMS farms and boxes has dropped. The same development can be seen in Sweden with the number of farms with AMS dropping for the first time in 2015. This can be explained by the ongoing structural development in Denmark and Sweden where some big farms with AMS, with many boxes, have been changing from AMS to conventional milking systems. Furthermore, the impact of low milk price has hit farmers hard in Denmark, Sweden and Finland in 2015. This can also be seen in the AMS data with decreasing numbers of AMS in Denmark and Sweden and the lowest increase in the number of AMS farms in Finland since 2003. The main reason might be the big difference in dairy farm sizes within the Nordic countries with Denmark having 177 cows per farm, Sweden with 81, Iceland with 43, Finland with 36 and Norway with 25 cows on average.

The main reason might be the big difference in dairy farm sizes within the Nordic countries with Denmark having 177 cows per farm, Sweden with 81, Iceland with 43, Finland with 36 and Norway with 25 cows on average.

Milk production from all the farms with AMS in the Nordic countries in 2015 has been estimated to stand at 3.718 million kg or 30,3% of total production, with 27,3% of the cows being milked with an AMS. Despite the high ratio of both milk and cows only 18,0% of the dairy farms in the Nordic countries have an AMS. The average size of the dairy herd on farms with AMS is estimated to be 89 cows and is much bigger than farms with conventional milking systems at 52 cows per farm.

It has been estimated that today over 30.000 farms worldwide use AMS, about 15% of those farms are within the Nordic countries.

Graph 1. Number of farms with AMS within the Nordic countries, 1996-2015



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WHAT DRIVES THE RE-EMERGENCE OF *STREPTOCOCCUS AGALACTIAE* AS CAUSE OF BOVINE MASTITIS?

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Introduction

Streptococcus agalactiae was the most common cause of bovine mastitis in Europe in the 1950s and is still highly prevalent in other parts of the world. Since the 1960s, implementation of mastitis control programmes has dramatically reduced its prevalence in Europe. In Northern Europe, however, *S. agalactiae* has recently made a come-back. The major components of the epidemiological triad that could drive this re-emergence are changes in the host, the pathogen or the environment. In this study, we investigate the hypothesis that re-emergence of *S. agalactiae* is driven by changes in the pathogen. Specifically, we postulate that bovine-adapted clones of *S. agalactiae*

dominated in the 1950s and 1960s, that those clones were largely eradicated in subsequent decades, and that re-emergence in Northern Europe is driven by spill-over of human-adapted clones into the bovine population.

Methods

To test this hypothesis, we examined a collection of 128 bovine *S. agalactiae* isolates from Sweden spanning 6 decades (1953 to 2012). Isolates were cultured from milk samples from individual animals with clinical or subclinical mastitis. With a few exceptions, a single isolate per herd was included, and herds were situated in different parts of the country. Isolates were initially characterized by multi-locus sequence typing, with in-depth analysis based on whole genome sequencing. Genomic DNA was extracted using the MasterPure Gram Positive DNA Purification Kit. Illumina library preparation was followed by Hi-seq sequencing using standard protocols (Illumina, Inc, USA). Phylogenetic trees will be estimated with RAxML v7.0.440 for all sites in the core genome containing SNPs, using a GTR model with a gamma correction for among site rate variation and ten starting trees. As part of this process, data will be partitioned into synonymous, non-synonymous and intergenic SNPs as this has been shown to improve likelihood estimates. Support for nodes will be assessed using 100 random bootstrap replicates. Further analysis using Gubbins and Roary will allow recombination events to be identified and mobile genetic elements to be characterised.

Results and Discussion

Preliminary results show that recent isolates (2010-2012, n = 45) primarily belong to ST1 (24%), ST103 (18%), ST196 (13%) and ST23 (11%). None of these types are related to the bovine-adapted clonal complex that was described in the UK and that is hypothesized to represent the dominant European type of *S. agalactiae* in the 1950s. ST1, ST23 and ST196 have been associated with carriage and disease in humans, indicating that people may serve as a source of infection for cattle, or vice versa. ST103 is common in cattle in China and may be associated with environmental transmission. Whole genome sequencing of the entire collection will provide additional insight into the composition of the *S. agalactiae* population, changes in this population over the time, and the role of lateral gene transfer of host-adaptation genes such as the lactose operon.

Importance

This study will help to understand the role of bacterial evolution in the re-emergence of *S. agalactiae* and will provide an indication of the risk of zoonotic or anthroponotic transmission.

NOTES

PRACTICAL APPLICATION OF ON FARM CULTURING

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SUMMARY

On farm culture has received increasing attention over the last years, because of the constant focus on consumption of antibiotics for intermammary treatment and cost cutting. Farmers can benefit with the application of a simple and easy-to-use system for selecting treatment/non treatment. Based on small scale preliminary experience a larger ongoing project was founded by the Danish Milk Levy. The aim of the project was to gain experience with on farm culturing and as a side effect reduce the number of mastitis treatments and secondarily the use of antibiotics. Material was developed for training purposes for both the herd veterinarian and the farmer. Initially a kick-off workshop was held, presenting the material and some hands-on experience working with the Selma Plus agar plates. Next, the veterinarians trained the farmers and made a regular follow-up on sampling, culturing and decision-making. Culture positive plates were sent to the Danish Veterinary Institute for verification with Maldi-Tof.

INTRODUCTION

In recent years the interest in on farm culture have increased worldwide, and different selective and easy to read agar plates have been tested.

The application has been for various reasons; reduction in the consumption of antibiotics, targeting the treatment, and an alternative to no diagnostics on some farms. A study from the US was conducted in 2011 (1), to evaluate possible reduction in the use of antibiotics, withholding time and bacteriological outcome. It reduces the intermammary treatment by half, without significant differences in clinical cure and bacteriological cure risk.

In a follow-up study with focus on additional outcome from 2011 (2), the long term outcomes such as; recurrent clinical mastitis in the same quarter, SCC, milk production and survival, resulted in no difference after application of the result from the on farm culture system.

In the US, 3M Petrifilm and the Minnesota Tri – plate system are popular choices. When considering the sensitivity to *Staph. aureus* the Minnesota Culture System was the best option (3) on laboratory conditions.

In Scandinavia the SELMA Plus® system has been used in recent years. The agar is a four partioned agar plate; bovine blod agar with esculin,

MacConkey agar, mannitol salt agar and PGUA agar for identification of *E. coli*.

The Danish interest in this system is a combination of cost cutting and the very poor availability of fast cost efficient diagnostics services. At the same time the farms are increasing in size (185 cows in average August 2016), the same for management level and demand on know-how. Therefore the author has implemented the system on selected farms, with a high management level in 2010 to 2014. In 2016 an application was sent to the Danish Milk Levy Fund, to further study the concept and develop guidelines to veterinarians and farmers.

ON FARM CULTURE IN PRACTICE

The system is centered on a web-page with information for veterinarians and a section of targeted dairy farmers. It is a combination of SOP's, literature, instruction videos and easy-to-read manuals.

We are very clear in the presentation of the material that it does not replace professional diagnostic lab work and it is essential to implement and maintain it in close cooperation with the herd veterinarian.

The project was divided into three steps:

- Train the trainer
- Train the farmer
- Follow-up diagnostics at the Danish Veterinary Institute

The project began with a kick-off workshop with brush-up on practice diagnostics for veterinarians. Scientific staff from the Danish Veterinary institute and international experts participated, sharing their knowledge with the participating practitioners.

The material developed for both veterinarians and farmers was discussed, and a hands-on workshop in the lab reading the Selma Plus agar plates was part of the workshop as well.

Each vet participated with 2-3 farms during a four month project period. The dairy farmer receives training from the veterinarian and continuously follow-up at the weekly herd check.

The farmer is instructed to grade the clinical case of mastitis in three different grades (4), before collecting a sample. They are then going to plate it, and read and record growth/no growth after 12-24 hours.

The aim of the project is to reduce the prevalence of treatment, to reach the goal of reducing the consumption of antibiotics back to the 2012 level in late 2018. Next is an evaluation of the robustness in the system and practical feedback from both farmers and veterinarians.

Each plate with a positive culture is sent to the Danish Veterinary institute for follow-up diagnostics with Maldi-Tof. This is to evaluate the diagnostic and also to follow-up on the decision related to the culture. Due to practical reasons the project period is short and the data collected will be limited.

CONCLUSIONS

The application of on farm culture can be a tool in reducing the number of treatments and therefore the consumption of antibiotics. The system naturally has its limitation, because farmers are not trained in working with microbiology. But through close cooperation with the herd veterinarian and follow-up diagnostics, it can be applied successfully at a farm with a high management level, according to the literature.

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IMMUNE RESPONSE INDUCED BY A VACCINE ASSEMBLY OF RECOMBINANT PROTEINS OF *STAPHYLOCOCCUS AUREUS* AND DNA VACCINE: A STUDY IN A MOUSE MODEL

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Bovine mastitis is one of the most common and costly disease for the dairy production chain worldwide. Among several microorganisms that cause bovine mastitis, *Staphylococcus aureus* remains as one of the most significant mastitis pathogen. In face of, the development of vaccines for protection from new intramammary infections (IMIs) by *S. aureus* is of considerable interest (1). Moreover, the use of vaccines that employ new technologies such as DNA vaccine and recombinant protein vaccines for the virulence factors of this bacterium have been suggested (1,2). The immune protection induced by most current vaccines seen to mediated by long-live humoral immune response. Otherwise, the development of vaccines against *S. aureus* has consistently failed, in which the cellular immune response is crucial in mediating protection (3).

Thus, this work was designed to evaluate the immunogenicity of three recombinant proteins of *S. aureus*, associated or not with a DNA vaccine in mice in order to subsequently elaborate an efficient vaccine against mastitis by *S. aureus*. For this purpose, the recombinant proteins were produced using a prokaryotic expression system. The DNA vaccine comprised the plasmid pCI-neo mammalian expression vector encoding the co-stimulatory factor gene. Immunizations were performed using nine experimental groups with seven mice C57bl/6J per group, as follows: G1) protein A; G2) protein B; G3) protein C; G4) protein A, B and C; G5) protein A, B and C and the co-stimulatory molecule; G6) protein A and the co-stimulatory molecule; G7) protein B and the co-stimulatory molecule; G8) protein C and the co-stimulatory molecule; G9) control group. Here, due to a conflict of interest (patent fillings), the description of the recombinant proteins and the DNA vaccine for the co-stimulatory molecule were not showed. The DNA vaccine (10 µg) for the co-stimulatory molecule was the prime immunization, followed by three doses containing the recombinant proteins (20 µg per protein) using the adjuvant saponin with intervals of 14 days. The euthanasia of the all animals were performed after 64 days of experiment (21 days after the last vaccination). Then, the splenic lymphocyte proliferation response in cell culture unstimulated or stimulated by γ irradiation inactivated *S. aureus* strain

isolated from a persistent bovine IMI was performed by the uptake of 5'-bromo-2'-deoxyuridine (BrdU). The immunophenotyping of the spleen cell culture (5% CO₂, at 37 ° C for 120 h) unstimulated or stimulated by the inactivated *S. aureus* strain was analyzed by flow cytometry using monoclonal antibodies to determine the percentage of T cells (CD3⁺) and their subpopulations (T CD4⁺, T CD8⁺ and T $\gamma\delta$) and main memory subsets: central (CD44^{high}CD62L^{high}) and effector (CD44^{high}CD62L^{low}) memory cells.

The animals from the G5 group revealed the most interesting outcomes. For instance, the most pronounced results of G5 group were the highest lymphocyte proliferation index, and the highest percentage of T $\gamma\delta$ CD44^{high} CD62L^{low} ($P \leq 0.05$). Furthermore, a decrease in the percentage of T $\gamma\delta$ and T $\gamma\delta$ CD44^{high} CD62L^{high} in *S. aureus* stimulated cell culture in the control group was observed when compared with unstimulated cell culture ($P \leq 0.05$) that was not found in G5. These results should be highlighted specially regarding the crucial importance of T $\gamma\delta$ cell subpopulations in the protection against *S. aureus* subsequent infections (3).

It was concluded that the DNA-recombinant proteins vaccination strategy (G5 group) generated a promising cellular immune response toward an effective vaccine against *S. aureus* mastitis.

ACKNOWLEDGEMENT

The study was supported by Conselho Nacional de Pesquisa Científica e Tecnológica (CNPq; Project n. 480102/2011-2). HG, MAG, APL, MAVPB, AMMPDL, FGF, MBH are indebted to CNPq for their fellowships.

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IDENTIFICATION OF POTENTIAL MARKERS IN BLOOD AS A RISK FACTOR FOR OCCURRENCE OF HIGH MILK SOMATIC CELL COUNTS IN DAIRY COWS DURING THE TRANSITION PERIOD

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A broader approach and a better understanding of the underlying conditions of mastitis can help us to establish strategies to diagnosis and control this disease. Notably, the metabolic changes during the transition period may predispose cows to various diseases (Rezemand et al., 2007; Moyes et al., 2009), and among them, we highlighted the intramammary infections.

Thus, the objective of this work was to determine if there are associations between blood parameters and the occurrence of high milk SCCs in dairy cows during the transition period. Here, we used 34 Holstein cows that were aleatory divided into three groups: cows that received antimicrobial (CEPRAVIN[®], MSD, Brazil) treatment in all quarters at drying-off (n = 10 cows, 40 quarters), cows that received internal teat sealant (TEAT SEAL[®], Zoetis, Brazil) in all quarters at drying-off (n = 11 cows, 44 quarters), and control untreated dairy cows (n = 13 cows, 52 quarters). We collected quarter milk samples and blood samples at drying-off (60 days before the expected day of parturition; M1), at parturition (M2), and three (M3), seven (M4), 15 (M5), 21 (M6) and 30 (M7) days after calving. Blood samples were collected for determination of the leukogram (total leukocytes count, and the percentage and the absolute values of neutrophils, eosinophils, basophils, monocytes and lymphocytes), hemogram (total erythrocyte count, hemoglobin concentration, hematocrit, platelet count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and mean corpuscular volume), and the serum concentration of calcium, phosphorus, total protein, albumin, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, beta-hydroxybutyrate (BHBA), non esterified fatty acids (NEFA), urea and creatinine, and the activities of alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase, and the plasma concentration of fibrinogen.

The variables under examination were first analyzed individually to verify the significance, and then in combination to assess the effect of the single variables on all others. In the first stage of the analysis, unconditional logistical model for each variable related to the high SCCs which *P*-values ≤ 0.20 were considered as a select variable passed to the next stage of the analysis. In the final model variables selected in the first stage were used to develop a final model in a multivariate logistical model with *P* ≤ 0.05 considered to be retained in in the final model.

A logistic regression model was adjusted, taking the following as explanatory variables ($P \leq 0.20$): treatment, moment, hematocrit, mean corpuscular hemoglobin concentration, urea, creatinine, LDL, total protein, fibrinogen and albumin. In this model, it was found that only some variables were statistically significant ($P \leq 0.05$) for estimating the likelihood of having high milk SCCs, and these remained in the final model, as following: antimicrobial treatment (odds ratio = 0,258, $P = 0.002$), M3 (odds ratio = 0,041; $P = 0.005$), M4 (odds ratio = 0.015, $P \leq 0.0001$), M5 (odds ratio = 0.02; $P = 0.001$), M6 (odds ratio = 0.02, $P = 0.001$), M7 (odds ratio = 0.02, $P = 0.001$), total protein (odds ratio = 5.084, $P = 0.003$), fibrinogen (odds ratio = 1.001, $P = 0.04$) and albumin (odds ratio = 4.307, $P = 0.03$) contents, interaction between total protein and albumin (odds ratio = 0.804, $P = 0.02$). The interaction between total protein and albumin concentrations indicates that the total protein content depends on, at least in part, albumin concentration. Thus, the interaction between albumin and total protein contents should be considered as a protective effect (odds ratio < 1.0). Firstly, when analyzed individually in the logistic regression model, the albumin – a negative acute phase protein, was negatively associated with milk high SCCs (odds ratio = 0.888, $P = 0.06$), in contrast to total protein content that was positively associated with high SCCs (odds ratio = 2.121, $P = 0.0001$). In face of, the discrepancy between serum total protein and albumin contents in the logistic regression model may be due to the globulin content, which most of their fractions are comprised by positive acute phase proteins.

Although, some widely studied variables related to metabolic disorders during the transition period, such as BHBA, NEFA and glucose (1, 2), were not eligible to enter in the logistic regression model to identify which blood markers are related to high milk SCCs, others variables that can be easily and quickly determined under field conditions, such as the measurement of total protein, were associated with high milk SCCs.

ACKNOWLEDGEMENT

The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Project n. 2012/08982-4). AMMPDL is indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for her fellowship. KRS and FNS are indebted to FAPESP to their fellowships.

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PROCESS CONTROL IN PLATFORM MOUNTED TEAT DISINFECTION SYSTEMS

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Post milking teat disinfection has been an important component of a mastitis control programme for over 50 years. Adoption of the technique, based on recommendations contained within the NIRD Five Point Mastitis Control Plan, played a significant part in the reduction of mastitis infections caused by the contagious mastitis pathogens.

The increased prevalence of environmental mastitis infections has seen the widespread adoption of pre-milking teat disinfection.

Post milking application of a teat disinfectant fulfils two roles. Firstly, to apply disinfectant to the teat end and barrel in order to disinfect all teat surfaces and destroy any bacteria present, soon after the cluster is removed from the cow. Secondly, to apply skin conditioning products to ensure the teat remains soft and supple and is able to cope with the rigours of machine milking. Poor teat coverage, with a post milking disinfectant, can lead to a reduction in teat skin condition, an increase in bacterial colonisation of the dry surfaces and adversely affect the ability to clean the teat.

For these objectives to be achieved, a teat disinfectant product must be applied in a timely fashion together with good coverage of the teat end and teat barrel. A study carried out in 2013 on 10 commercial dairy farms set out to assess the effectiveness of manual post milking teat disinfection using a hand operated vacuum spray lance against the objectives of barrel and teat end coverage. On average, 3.77 teat ends out of a possible 4.00 received teat disinfectant (94.0%). On average, 50.3% of the teat barrel surface received teat disinfectant. There was considerable farm to farm variation (19.8- 83.4% coverage) highlighting the variability of operators.

In an attempt to reduce variation and apply some process control to the activity, an evaluation was carried out using a rotary platform mounted teat disinfection system (Ambic Equipment Ltd Locate'n'Spray™). Teat coverage was assessed on two occasions with teat end coverage measured at 96.0% and teat barrel coverage measured at 91%. These results are significantly improved compared with manual teat spraying.

This confirms that an automated system for applying teat disinfection, in a timely fashion after the cluster is removed, is capable of applying teat disinfectant more accurately and more consistently than a human operator with a vacuum operated teat spray lance.

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PROVIDING PRODUCERS WITH INFORMATION TO MAKE INFORMED DECISIONS ON SDCT

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With an ever increasing number of ‘figures’ and ‘datapoints’ for producers to refer to making the information accessible and informative has to be the key for any service provider.

This element of the right data for the right purpose sits alongside wider industry needs to evidence how the sector acts both proactively and reactively. The on-going focus on Antimicrobial Resistance has translated into the dairying sector taking a closer look at the different types and timings of antibiotic use. It is well known across the industry that diseases prevention is better than cure but for those times when infection is present it is important that the appropriate medicine is available to the industry.

As a service provider Cattle Information Services are acutely aware of the needs of producers for clear and usable data to aid with decision making – it with this in mind that all reports from the companies YourHerd management programme are designed.

In respect of data that producers are asking for when cows are approaching their dry period we reacted to requests from producers to not only show the date the animal is due to calve and her latest somatic cell count (SCC) test data but also the preceding three tests and total number of tests above the 200 threshold.

Displaying this data on the action list of cows due to dry helps producers make more informed decisions when determining which cows would benefit from treatment at the start of the dry period – true selective dry cow treatment based on evidence.

ACUTE PHASE PROTEINS PROFILE OF MILK IN MULTIPLE PATHOGEN INFECTIONS

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Severity and course of mastitis is largely determined by the mastitis causative pathogen. This can be further impacted by the presence of multiple species within the affected gland. This pathogen effect is also reflected in onset and level of release of inflammatory markers such as acute phase proteins (APP) into milk during mastitis (1-3). It is thus important to consider the APP profile in milk during mastitis when multiple pathogens are involved in the intramammary infection leading to the inflammation. This study aimed to determine the profile of two APP; mammary associated serum amyloid A3 (MSAA3) and haptoglobin (Hp), in milk samples in which a polymerase chain reaction (PCR) analysis identified multiple mastitis pathogens.

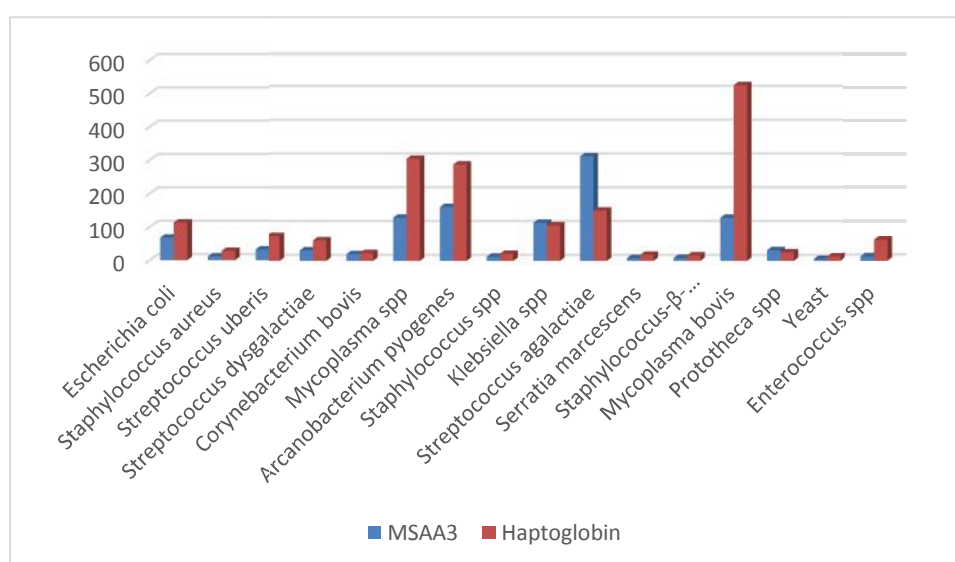
Milk samples, (n=137), from cows with clinical mastitis from several farms across England, submitted to the Westpoint Veterinary group diagnostic laboratory, for mastitis diagnosis were subjected to a real time multiplex PCR assay (PathoProof Mastitis PCR Assay kit, Thermo Fisher Scientific, Espoo, Finland), with a target of 16 pathogens (primers). Haptoglobin and MSAA3 were measured in the same samples using an in-house ELISA for Hp and a commercial ELISA kit (Tridelta Development Co. Kildare, Ireland) for MSAA3.

All 16 pathogens (primers) were detected in the samples. Thirty samples had none or only one pathogen-type detected in them. Two pathogens types were detected in 41 of the samples, 3 in 37 samples, 4 in 22 samples and 5 in 6 samples. Samples in which more than 3 pathogen species were detected could be considered as contaminated according to Kalmus et al. (2013); however, APP values for such samples were still computed and presented. The APP concentrations in studied milk samples ranged from <0.4 to 1049 µg/ml with a median of 29.92 µg/ml (Hp) and <0.6 to 1500 µg/ml with a median of 12.43 µg/ml (MSAA3). No significant differences were found in the APP based on number of pathogens detected in the sample (P=0.07), rather, the APP were significantly different among samples with specific species (irrespective of number of pathogens co-detected); *Mycoplasma* spp, *Arcanobacterium* spp, *Streptococcus agalactiae* *Escherichia coli*, *Streptococcus dysgalactiae* and *Streptococcus uberis* showed the highest levels of APP (in descending order). The observation that specific pathogens showed significant differences in APP values corroborates previous findings (1-2). The most frequently identified pathogen was *Staphylococcus* spp (detected in 110 samples) suggesting that it could be a frequent

contaminant of the samples. Since no significant variations were observed in the APP of samples having more than one pathogen-species identified, it may be concluded that only a particular pathogen out of all detected in the samples, was actively involved in the pathogenesis of mastitis, while others were merely contaminants.

Using PCR to detect mastitis causing pathogens has an advantage of added sensitivity and speed over the conventional microbiological culture and isolation; however, a setback with this method is that often, multiple pathogens are detected in a single sample as seen in this study. This raises the need to determine the level of involvement of each detected pathogen in the mastitis stimulation. Knowing the characteristic of APP in milk may be beneficial as APP have been established to vary significantly in mastitis depending on the causative pathogens (Figure 1).

Figure 1. Acute phase proteins (MSAA3 and Hp, $\mu\text{g/ml}$) in milk samples (n=107) with multiple species detected; medians across presence of particular pathogens



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PEGBOVIGRASTIM: A HISTORY OF THE FUTURE

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INTRODUCTION

Bovine granulocyte colony stimulating factor (bG-CSF) naturally increases the number and function of neutrophils in dairy cows. When administered at calving, a time when cows typically experience innate immune suppression, it has been shown to increase the number, myeloperoxidase (MPO) function, L-selectin activity and direct migration of neutrophils (Kehrl M.E., 1991) (Kimura K., 1999). Polyethylene glycol-bound bG-CSF (pegbovigrastim), also has been shown to increase neutrophil numbers and MPO release (Kimura K., 2014). A key on-farm parameter chosen to investigate the efficacy of pegbovigrastim was the incidence of clinical mastitis in the first 30 days post calving, due to its common nature, economic impact on dairies and the reliability of recording.

STUDY DESIGN

In a series of experiments, pegbovigrastim was administered subcutaneously to dairy cows at doses of 5, 10, and 20 μ /kg, targeted at day -7 and the day of calving. Controls were administered an equivalent amount of saline solution. The incidence of mastitis in the first 28 days after calving (Hassfurth R.L., 2015) were measured.

RESULTS

Pegbovigrastim significantly reduced the incidence of clinical mastitis in the first 28 days in milk at doses of 10 and 20 μ g/kg (50% and 72% respectively) (Table 1, Hassfurth R.L., 2015).

SUMMARY AND INTO THE FUTURE

Subcutaneous administration of pegbovigrastim aiming at 7 days before and within 24 hours after calving has been shown to restore the number and function of neutrophils, and is effective at reducing the incidence of clinical mastitis in early lactation as anticipated from its mode of action.

There is further scope to research other effects of immune modulation by pegbovigrastim.

Table 1. Showing reduction in incidence of clinical mastitis in first 28 days in milk at varying doses of pegbovigrastim (Hassfurth R.L., 2015)

Group	Number (%) of clinical mastitis cases	% Reduction
Saline solution (n=53)	18 (34%)	
5µg/kg (n=51)	10 (19.6%)	44%
10µg/kg (n=54)	9 (16.7%) p=0.044	50%
20µg/kg (n=53)	5 (9.4%) p=0.004	72%

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RELATIONSHIP BETWEEN MILK CATHELICIDIN ABUNDANCE AND MICROBIOLOGIC CULTURE IN CLINICAL MASTITIS.

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The availability of reliable tools enabling a sensitive and specific detection of mastitis in dairy cows can significantly aid control strategies and promote a more rational use of antimicrobials. Recently, we developed a milk cathelicidin ELISA showing an elevated sensitivity and specificity for dairy cow mastitis (1). Cathelicidin is an antimicrobial and immune defense protein that is specifically released in milk following a microbial stimulus. In 2011, a study carried out by Smolenski and coworkers on a limited number of cows suggested that cathelicidin abundance in milk might be influenced by the mastitis-causing pathogen (2). In addition, these authors reported that a percentage of the milk samples did not show any cathelicidin reactivity by western immunoblotting. To evaluate the possible impact of the mastitis-causing microorganism on the diagnostic value of our cathelicidin ELISA, we carried out a study on the milk of cows with clinical mastitis caused by different microorganisms. A total of 535 quarter milk samples were investigated. Of these, 435 were collected from quarters showing signs of clinical mastitis, while 100 were collected from healthy quarters, had less than 100,000 cells/mL and were negative to microbiologic culture. The latter were used as a negative control. All samples were subjected to milk cathelicidin ELISA, somatic cell counting, and microbiologic culture.

Of the 435 clinical mastitis samples, 431 (99.08%) were positive to the cathelicidin ELISA, 424 (97.47%) had SCC >200,000 cells/mL, and 376 (86.44%) were positive to microbiologic culture. Given that almost all samples collected from clinically affected cows were positive to the cathelicidin ELISA, the abundance of cathelicidin and the extent of SCC increase did actually change depending on the causative agent (Figure 1). We did also observe differences in behavior between cathelicidin and SCC depending on the pathogen; *Streptococcus agalactiae* induced the highest cathelicidin abundance, while *Serratia* spp. induced the highest SCC. On the other hand, *Serratia* spp. induced the lowest cathelicidin increase, while CNS induced the lowest SCC (Table 1). Nevertheless, The different ability of the microorganisms to induce cathelicidin release in the milk did not compromise its value as a mastitis marker. The highest sensitivity was observed for the cathelicidin ELISA (99.1) in comparison to SCC (97.5) or to microbiologic culture (86.4). The 100 samples collected from healthy cows were all negative to the cathelicidin ELISA, corresponding to a 100% specificity in the evaluated sample cohort.

In conclusion, this study confirmed the value of our milk cathelicidin ELISA for detecting bovine mastitis, and highlighted an influence of the mastitis-causing microorganism on cathelicidin abundance. Such influence does not

compromise diagnostic performance, but upon further investigation it might have potential for indicating disease severity and evolution, especially when considering the close connection of cathelicidin with the polymorphonuclear neutrophil (PMN) component of milk somatic cells.

Figure 1. Boxplots illustrating the distribution of cathelicidin and SCC for the different microorganism classes in the 435 clinical quarter milk samples.

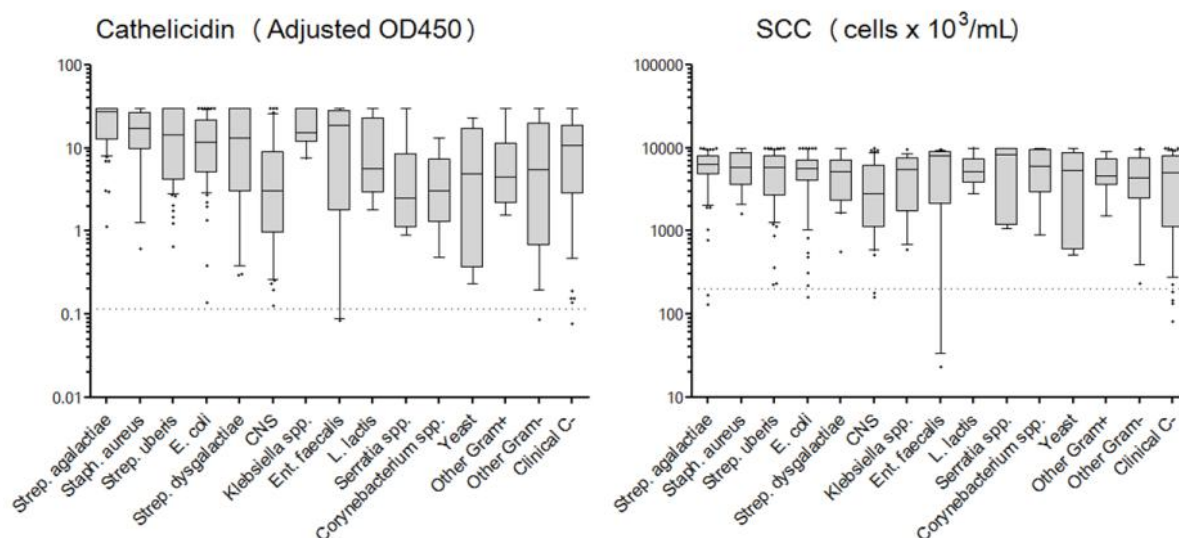


Table 1. Median and interquartile ranges of cathelicidin and SCC observed for the different microorganisms in the 435 clinical quarter milk samples. Microorganism classes are reported in decreasing order according to the respective median value

Sample class (N)	Cathelicidin (AOD450)		Sample class (N)	SCC (cells x 10 ³ /mL)	
	Median	Interquartile Range		Median	Interquartile Range
Streptococcus agalactiae (64)	27.565	12.290/30.000	Serratia spp. (8)	8,183	1,151/9,731
Enterococcus faecalis (13)	18.540	1.743/28.285	Enterococcus faecalis (13)	7,982	2,092/8,966
Staphylococcus aureus (18)	17.070	9.572/26.505	Streptococcus agalactiae (64)	6,452	4,688/7,947
Klebsiella spp. (17)	15.350	11.795/30.000	Corynebacterium spp. (7)	6,029	2,859/9,483
Streptococcus uberis (72)	14.350	4.115/30.000	Staphylococcus aureus (18)	5,821	3,569/8,663
Streptococcus dysgalactiae (29)	13.070	2.941/30.000	Streptococcus uberis (72)	5,812	2,645/8,137
Escherichia coli (65)	11.610	4.985/21.915	Escherichia coli (65)	5,588	3,950/7,252
Culture negative (59)	10.620	2.803/18.920	Klebsiella spp. (17)	5,526	1,719/7,572
Lactococcus lactis (9)	5.620	2.888/23.355	Other Gram-positives (8)	5,468	2,987/7,275
Other Gram-negatives (18)	5.540	0.660/20.162	Yeast (6)	5,318	600/8,894
Yeast (6)	4.869	0.364/17.062	Lactococcus lactis (9)	5,209	3,751/7,275
Other Gram-positives (8)	4.543	2.177/11.582	Streptococcus dysgalactiae (29)	5,126	2,309/7,108
CNS (42)	3.050	0.938/8.977	Culture negative (59)	5,049	1,106/7,992
Corynebacterium spp. (7)	3.040	1.275/7.310	Other Gram-negatives (18)	4,328	2,440/7,541
Serratia spp. (8)	2.474	1.092/8.445	CNS (42)	2,808	1,091/6,144

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DIAGNOSTIC PERFORMANCE OF A MILK CATHELICIDIN ELISA FOR MASTITIS DETECTION

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Adding to the currently established somatic cell count (**SCC**) and bacteriologic culture (**BC**), the identification of inflammation-specific protein markers can open the way to the development of immunoassay-based screening strategies enabling a more sensitive and reliable mastitis monitoring. Cathelicidin is an antimicrobial and pro-inflammatory protein that is abundantly released by neutrophils and epithelial cells in milk following a microbial stimulus. We identified cathelicidin as a promising mastitis marker after an in-depth proteomic characterization of milk from ewes with natural and experimental intramammary infections by *Mycoplasma agalactiae* and *Streptococcus uberis*, respectively (1,2). Based on these studies, and after a large scale validation by western immunoblotting, we have developed a sandwich ELISA for cathelicidin detection in milk. The performance of our assay in detecting mastitis has been evaluated in dairy ewes and in dairy cows, and compared to SCC and BC.

Ewes

We collected and tested a total of 705 half-udder milk samples from Sarda ewes belonging to 3 flocks enrolled in a project for improvement of sheep mammary health carried out in the Island of Sardinia, Italy. As a result, 35.3% of samples (249/705) were positive for the cathelicidin ELISA. Microbiological culture was positive in 20.6% (145/705) of samples, with coagulase-negative staphylococci (**CNS**) being the most frequent finding (74.5%, 108/145). Cathelicidin abundance increased with the SCC. Accordingly, cathelicidin-negative (n=456) and cathelicidin-positive (n=249) sample classes clustered around SCC median values of 149,500 and 3,300,000 cells/mL, respectively. A strong correlation of cathelicidin with SCC was also seen based on the Receiver Operating Characteristics (**ROC**) curve analysis, with an Area Under the Curve (**AUC**) of 0.94 for cathelicidin against an SCC > 500,000 cells/mL. The AUC of cathelicidin against BC was 0.76. Since neither SCC nor BC can be considered as gold standards for the true disease status, sensitivity (**Se**) and specificity (**Sp**) of the cathelicidin ELISA were assessed with a Bayesian cross-validation approach based on the Latent Class Analysis (**LCA**). When considering a threshold of 500,000 cells/mL, Se was 92.3% for cathelicidin, 89.0% for SCC, and 39.4% for bacteriology, while Sp was 92.3% for cathelicidin, 94.9% for SCC, and 93.6% for bacteriology (3).

Cows

We collected and tested a total of 531 quarter milk samples from Holstein cows belonging to two herds. As a result, 29.0% of samples (154/531) were positive for cathelicidin, 18.8% (100/531) had SCC > 200,000 cells/mL, and 13.7% (73/531) were positive for BC. The AUCs following ROC curve analysis against BC were 0.78 and 0.75 for cathelicidin ELISA and SCC > 200,000 cells/mL, respectively. Cathelicidin showed a strong correlation with SCC. Accordingly, cathelicidin-negative and cathelicidin-positive samples clustered in association with low and high SCC values, with median values of 3,000 and 286,000 cells/mL for cathelicidin-negative and for cathelicidin-positive samples, respectively.

Based on LCA, the cathelicidin ELISA showed a higher Se than both SCC and BC. Cathelicidin Se was 6.2 percentage points higher than SCC > 200,000 cells/mL (80.6% *vs* 74.4%, respectively), reaching levels even higher than SCC > 100,000 cells/mL (80.6% *vs* 80.2%, respectively). Most importantly, this was counterbalanced by a loss in Sp of only 1.4 percentage points (94.9% Sp for cathelicidin *vs* 96.3% Sp for SCC > 200,000 cells/mL). The limited Se of BC was confirmed also in this study (38.8%). In addition, BC did also show a slightly lower Sp than both cathelicidin and SCC (4).

In conclusion, this study confirms that cathelicidin is released in milk of cows and ewes with mastitis, that its presence is strongly correlated to high SCC, and that its measurement by ELISA can improve sensitivity of mastitis detection without compromising specificity.

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CAN THE ICEQUBE™ BE USED AS AN EARLY ALERTING SYSTEM FOR CLINICAL MASTITIS?

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Mastitis in dairy cattle is a major cost to the dairy industry (Sargeant et al, 2001), resulting in increased veterinary treatment costs, reduced milk yield and increased culling rates (Sargeant et al, 2001). It is also considered to be a painful disease which has a negative effect on cattle welfare (Siivonen et al, 2011). Early treatment of the disease is important (Milner et al, 1996) and earlier detection of clinical cases may allow farmers and vets to intervene at an earlier stage. The objective of this study was to determine whether IceQube™ (IceRobotics, Edinburgh, Scotland) pedometers, attached to one hind leg of the dairy cow, could be used in conjunction with data from parlour software to accurately predict whether a cow is within two days of a clinical case of mastitis. A herd of 360 commercial Holstein dairy cows was studied over the period 1st February 2015 – 30th January 2016, 43 clinical mastitis cases (CMC) in 41 cows also had a complete IceQube & Fullwood Crystal (Fullwood Ltd, Ellesmere, Shropshire, England) dataset. IceQubes measured total lying and standing time, total number of lying and standing bouts, the minimum and maximum lying and standing bouts and the number of steps taken, on a daily basis. Additionally, the associated software also calculated a motion index (MI) and IceScore, which are functions of the aforementioned parameters. Fullwood Crystal milking parlour software was used to measure the daily yield, daily milking time, number of failed and yield carry over (YCO) milkings and conductivity of the milk. Cow activity changed significantly two days before a CMC, the number of steps increased (P=0.013), total lying time decreased (P=0.014) and conversely total standing time increased (P=0.03) and MI decreased (P=0.008). Parlour observations also changed significantly in the 2 days before a CMC: the daily yield decreased (P<0.001), daily milking time decreased (P=0.018), the number of YCO milkings increased (P<0.001) and conductivity increased (P<0.001). Due to collinearity, several parameters were excluded and the combination of daily yield, conductivity, number of steps and total lying time were found to accurately predict whether a cow was 2-1 days before a CMC or not (P=0.026). Therefore it was concluded that the IceQube, combined with data from Fullwood Crystal milking parlour software had good potential as an early detection system for CMCs within the study herd. Further work is needed to determine whether this is true across the wider dairy industry.

ACKNOWLEDGMENT

This study was completed as part of a MSc jointly funded by The Dairy Group and BBSRC.

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DEVELOPMENT OF A LATERAL FLOW POINT OF CARE TEST FOR THE RAPID DETECTION AND MEASUREMENT OF HAPTOGLOBIN IN BOVINE MILK

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SUMMARY

Haptoglobin (Hp) is regarded as a specific marker of mastitis, since the Hp concentration in milk from healthy quarters is low or undetectable (1) and Hp concentrations correlate closely to SCC (2). Hp can be measured by ELISA though this is impractical for large scale/ on farm analysis. As such this study aimed to determine the feasibility of a lateral flow assay (LFA) for the detection of Hp in milk for a point of care, 'cow side' diagnosis of subclinical mastitis (SM).

INTRODUCTION

Mastitis, inflammation of the udder, is the most common disease in dairy cows, affecting the yield, composition and processing properties of the milk, contributing to massive economic losses to the industry. The absence of any visible signs makes diagnosis of SM difficult (3,4) leading to delays in treatment and control of infections, allowing the spread of mastitis-causing pathogens and subsequent long-term reduction in milk yield (2,4). Somatic cell count (SCC) is the most common way to diagnose SM, though the sensitivity and specificity of the SCC in the diagnosis of mastitis are affected by factors not associated with udder inflammation (4). Haptoglobin, a specific marker of mastitis, can be measured by ELISA though this is impractical for large scale analysis meaning Hp, a highly sensitive biomarker of udder health and SC mastitis, is underutilised and restricted to research environments.

MATERIALS & METHODS

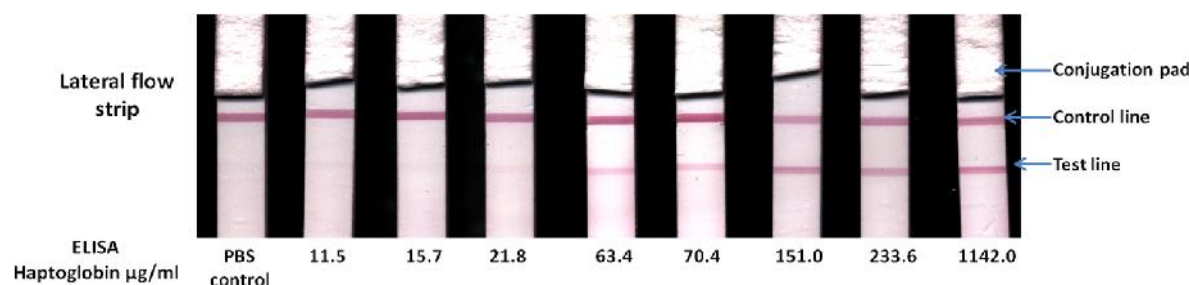
Test (rabbit anti-bovine Hp, Life Diagnostics) and control (rabbit anti-mouse IgG, Abcam) lines were applied 0.5mm apart onto UniSart® CN 140 nitrocellulose membrane (Sartorius) to form LFA test strips. Following a 2 hour drying at 37°C strips were attached to a backing card and an absorbance pad. Sheep anti-bovine Hp IgG (2), conjugated to colloidal gold was mixed with mastitic and normal milk diluted 1:50 in PBS and added to the test strips. Milk Hp was quantified using in-house ELISA as described previously (2).

RESULTS

A pink line was clearly visible line at the test and control lines for Hp positive (mastitic) milks samples. Only a control line was visible when using Hp negative (healthy) milk samples. Intensity of the test line correlates with

Hp concentration as determined by ELISA allowing for the development of a semi-quantitative test (Figure 1).

Figure 1. Lateral flow assay strips with milk samples of known Hp concentrations



DISCUSSION

Following development of the initial Hp LFA, further work to remove the dilution step (1:50), is necessary to avoid the 'Hook effect' associated with antigen excess. By removing this dilution step the Hp LFA can be used with greater ease on farm. The ease at which this test can be applied and the speed at which a result can be delivered mean that Hp LFA can be applied widely.

CONCLUSIONS

Haptoglobin is more sensitive than SCC but does provide a close correlation to SCC (2). Development and validation of a LFA for the detection and semi-quantification in milk and subsequent production of a point of care cow-side will allow this LFA to be undertaken on farm where applications can include detecting SC mastitis and may have a role in selective dry cow therapy.

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