BRITISH MASTITIS CONFERENCE

2014

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UNITED KINGDOM · CHINA · MALAYSIA

Topics:

- Mycoplasma
- RMS and green bedding
- Research updates
- Teat disinfection
- Role of vaccination in mastitis
- CNS mastitis

Wednesday 12th November 2014

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GENERAL INFORMATION

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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** *The* Dairy Group, UK
09:55	Mycoplasma mastitis – is it a problem in the UK?	**Ruth Zadoks** University of Glasgow, UK
10:25	Recycled manure solids and green bedding	**Andrew Bradley** University of Nottingham, UK
10.55	Questions and Discussion	
11:10	**COFFEE and POSTERS**	
	Research updates (also presented as posters)	**Brian Pocknee** *The* Dairy Group, UK
11:40	Evaluation of teat coverage with an automatic post milking teat disinfectant system using six different spray duration settings.	**Richard May** Ambic Ltd, Witney, UK
12.00	Best practices to prevent medicine residues in milk.	**Elizabeth Berry** BCVA, Gloucester, UK
12:20	Selective dry cow therapy using OrbeSeal.	**Judith Roberts** Zoetis, Tadworth, UK
12:40	Using medicines audits to monitor and drive responsible antimicrobial use in farm animal veterinary practice	**Kristen Reyher** University of Bristol, UK
13:00	**LUNCH and POSTERS**	
14:15	**WELCOME BACK AND VOTING ON POSTERS**	
	Session Three	**Elizabeth Berry** DairyCo, UK
14.20	Must know facts about teat disinfectants and their role in the prevention of mastitis in modern dairy herds.	**Mario G. López-Benavides** DeLaval, USA
14.55	The role of vaccination in mastitis control.	**Jonathan Statham** Bishopton Veterinary Group, Ripon, UK
15.30	Are Coagulase-Negative Staphylococci a problem?	**Sarne De Vliegher** Ghent University, Belgium
16.05	Questions and Discussion	
16:20	**POSTER AWARD and CLOSE**	
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Poster abstracts – also as an oral presentation in the Research Updates session

(presenting author underlined):

Appendix

CHAIRMAN'S INTRODUCTION

Welcome to the 2014 British Mastitis Conference.

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

Our first paper looks at the question of whether Mycoplasma is a problem in the UK. This is followed by a review paper on Recycled manure solids as a bedding for dairy cows. This paper brings the first session to an end.

Following the success in 2013 of the changed format for the R & D update, the scientific committee have again 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 a review of teat disinfection and its importance to udder health. This will be followed by a paper on the role of vaccination in the control of mastitis. The conference will be closed with a paper on the role of CNS and other minor pathogens in mastitis.

As always, I would urge you all to make time to review the posters and speak with the authors. Presenters each year put a great deal of effort into providing the abstracts and preparing and presenting their posters.

As previously we strive to find you the best speakers with the most relevant (and latest) information. This is achievable only thanks to all our generous sponsors. This year our sponsors are: Milk-Rite (Gold), Lely (Gold), Kilco (Silver), ADF Milking Ltd (Silver), Ambic Equipment Ltd (Bronze), Fullwood Ltd (Bronze), Ltd, Norbrook Laboratories (UK) Ltd (Bronze), Zoetis (Bronze) and Boehringer-Ingelheim (Bronze). As usual the event could not happen without able administration, provided by Karen Hobbs and Anne Sealey at *The* Dairy Group.

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

Ian Ohnstad
British Mastitis Conference Chairman
The Dairy Group

FURTHER INFORMATION

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

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What does NMC do?

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

A commitment to reducing mastitis and enhancing milk quality

Who are the members of NMC?

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

What can NMC do for you?

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

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

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

No other professional dairy organization enjoys the wide range of expertise found within the NMC membership.

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Since 1961, NMC has coordinated research and education efforts to help control the losses associated with mastitis. By bringing together all segments of the industry, a strong and successful organization has been created to enhance the quality of milk and dairy products. NMC welcomes your active participation and support. Please visit the NMC website for additional information and resources.

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MYCOPLASMA MASTITIS – IS IT A PROBLEM IN THE UK?

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SUMMARY

Mycoplasma mastitis has been known in the UK since the 1970s. With increases in herd size, the likelihood of detecting a *Mycoplasma* positive cow in a herd has increased. Detection can be based on culture of fresh bulk tank, composite or quarter milk samples or PCR of preserved milk samples. *Mycoplasma* can affect multiple organ systems and can be introduced through lactating cows, dry cows, heifers, calves and bulls. Vaccination and treatment are largely ineffective. Test and cull programmes have been implemented successfully in some herds, whilst other herds have eliminated *Mycoplasma* without such draconian measures. Prevention of contagious transmission is generally recommended, although evidence to support its effectiveness is limited. Environmental hygiene, e.g. cleaning and disinfection of stalls and bedding materials, may also have some impact. Poor hygiene around administration of intramammary treatments may lead to *Mycoplasma* mastitis. In large herds, staff training is important to ensure adequate hygiene during milking, treatment and in the cows' environment.

INTRODUCTION

Bovine mycoplasmosis, which includes respiratory disease, otitis, mastitis, arthritis and other disorders, is seen as an emerging infectious disease of dairy cattle in the EU (23). *Mycoplasma* mastitis has been known since the late 1970s, not only in the USA but also in the UK. Surveillance has not been sufficiently consistent to determine whether the prevalence of *Mycoplasma* mastitis has increased, although it often suggested that an increase in herd size is associated with an increased likelihood of finding a *Mycoplasma* positive animal in a herd (10, 25). Other authors have not found an association between herd size and *Mycoplasma* prevalence (12). Biosecurity, i.e. importation of cattle into the herd, may be more important than herd size per se (10). Multiple species of *Mycoplasma* may cause mastitis and multiple types of animals may be affected, including cows, bulls, young stock and calves, resulting in a variety of clinical presentations. Despite decades of descriptions, control efforts and research, *Mycoplasma* mastitis remains largely an enigma. We can detect *Mycoplasma*, we can speciate and strain-type it, we can take biosecurity and control measures, but it often seems to do as it pleases. In this paper, an overview of detection and control methods is provided with the aim to provide the practitioner with a menu of options for *Mycoplasma* mastitis control.

CLINICAL AND LABORATORY DIAGNOSTICS

Clinical Manifestations

Mycoplasma spp. have been identified as the cause of mastitis in lactating cows, dry cows, heifers and calves (9,14,19,29). Intramammary infection may be clinical or subclinical. In clinical cases, milk has been described as containing sandy sediment, with brown colouring and rice-like structure, although symptoms are variable not always specific (34). Quarters may be swollen but painless (28). In subclinical infections, the shedding level of somatic cells and *Mycoplasma* can be variable, which may lead to false-negative diagnostic results (4). The use of SCC to pre-screen cows prior to milk sampling for culture or PCR may lead to missed diagnosis, leaving a source of reinfection in the herd (25). In one outbreak study in the USA, 1,553 lacting cows were individually sampled to detect 88 positive cows, indicating the massive investment some people make in case finding (7). To ensure that a cow or a herd are truly negative, repeated testing is recommended. In some herds or animals, the initial manifestation of *Mycoplasma* infections is not as mastitis but as lameness, due to arthritis or to painful swelling of the mammary gland (29,36,37). Otitis media in pre-weaned calves has been described as the only visible abnormality in a herd with subclinical *Mycoplasma* mastitis (35). In some herds, *M. bovis* probably first occurred as respiratory tract pathogen of replacement calves (32).

Detection

Detection of *Mycoplasma* spp. is largely based culture or PCR. Culture can detect viable organisms, whereas PCR can detect DNA from viable and non-viable organisms. This is an advantage, because the viability of *Mycoplasma* spp. is compromised by transport, storage and freezing (5). Ideally, fresh samples are used for culture, which isn't always practical. PCR can be used on samples that have been frozen or preserved, which increases the sensitivity of the method. False-negative results may still occur, largely as a result of variable levels of *Mycoplasma* shedding by infected cows, but also when the PCR primers do not cover all relevant *Mycoplasma* spp. In early culture-based studies of *Mycoplasma*, the genus *Acholeplasma* was sometimes confused or grouped with the genus *Mycoplasma.* Because *Acholeplasma* is now largely considered non-pathogenic, most laboratories differentiate between the genera (3). A second advantage of PCR compared with culture is the faster turn-around: PCR takes hours, culture can take up to 10 days. Bulk tank monitoring is highly recommended for monitoring of herd status, with follow up at string, cow or quarter level when *Mycoplasma* is detected (10).

Species identification

The next level of diagnostic detail is identification at species level. Multiple species of *Mycoplasma* have been associated with mastitis in the UK, including *M. bovis*, *M. bovigenitalium, M. californicum*, *M. canadense*

(6,14,15,19). In other countries, additional species have been identified in milk, including *M. alkalescens, M. arginini, M. bovirhinis, M. capricolum* and *M. gateae* (13,17,25). *M. bovis* is more prevalent than any of the other species. In one case study from the UK, an outbreak of *M. bovigenitalium* was described as mild, and the outbreak was resolved without resorting to segregation and culling (14). By contrast, another outbreak in the UK, caused by the same species, was described as severe and 60 of 75 animals failed to return to normal despite various intramammary and systemic antibiotic treatments (9). Based on current knowledge, the different species are mainly important from a diagnostic perspective, particularly in relation to the specificity of PCR primers. The clinical or epidemiological relevance of species identification is largely unknown.

Strain typing

The third level of diagnostic detail is the strain level, whereby subpopulations within species are differentiated. Many methods have been used for this purpose, including RAPD, PFGE, AFLP, MLVA and MLST (21,31). A detailed explanation of these "four letter words" is not relevant here, but different methods are appropriate for different investigations (spatial and temporal scale). Strain typing has been used to study the within-animal, within-herd and between-herd epidemiology of *M. bovis*. Within a herd, multiple species of *Mycoplasma* may exist, but a single species and strain tend to dominate. Within an individual animal, multiple strains of *M. bovis* may co-exist, but again a single strain tends to predominate in the mammary gland and extra-mammary tissues. Strain typing has been used to trace the country of origin of *M. bovis* outbreaks attributed to imported cattle (2). In some European countries, e.g. Austria and Denmark, multiple outbreaks of mastitis spanning several years have been associated with a single predominant strain of *M. bovis* (18,30). In other countries, e.g. Switzerland, multiple strains have been identified, which argues recent importation of a particular strain as the cause of the emergence of *Mycplasma* mastitis (1). In the UK, two groups of strains have been observed but an association between strains and clinical, temporal or spatial origin has not been detected (21). Strain typing is primarily relevant for research and for a limited number of specific epidemiological questions.

CONTROL MEASURES

Prevention

Calves may become infected through feeding of waste milk from cows with high SCC or receiving antimicrobial treatment. Pasteurization of milk will eliminate this risk. Pre- and post-pasteurization culture can be used to assess the effect of pasteurization (8). PCR should not be used for this, because it does not differentiate between viable and dead organisms.

Transmission of *Mycoplasma* may occur via nose-to-nose contact and result in an outbreak of *Mycoplasma* mastitis, or it might occur during the milking time (10). To prevent introduction into the lactating herd via the respiratory route, veal calves and beef animals should be separated from dairy animals on mixed operations (36). To prevent transmission via the milking machine, purchased cows and heifers should be quarantined and tested for mycoplasmal mastitis before admission to the regular herd (10,11). Whilst it may seem like a good idea to separate cows with *Mycoplasma* mastitis from the rest of the herd by putting them in a hospital pen, this may lead to mastitis outbreaks among other animals in the hospital pen so it is better to separate them completely from other lactating animals (26).

Vaccination

This is one of the only things that are easy about *Mycoplasma:* to date, no effective vaccines have been described. Autogenous vaccines are offered by some companies, e.g. in the USA, but their efficacy is unproven. Vaccines don't prevent, decrease the incidence, or ameliorate the clinical signs of mycoplasmal mastitis (11).

Treatment

This is the second easy but unhelpful aspect of *Mycoplasma* mastitis. It has repeatedly been described as non-responsive to conventional antimicrobial treatment (9,28,34,37). Because *Mycoplasma* spp. lack a cell wall, they are inherently resistant to multiple antimicrobials. In addition, they have acquired resistance to a number of compounds, including tetracycline, oxytetracycline, lincomycin, spectinomycin, tilmicosin and tylosin (24,33). In some older studies, unhygienic administration of intramammary treatment has been identified as a risk factor for *Mycoplasma* mastitis outbreaks (12,19). Although this shouldn't be necessary with modern, sinlge-use intramammary products, it is conceivable that issues with staff training or turn-over in large herds could lead to "re-emergence" of this risk factor. *Mycoplasma* arthritis is generally also unresponsive to treatment

Eradication

To start with the good news, there are many examples of successful eradication of *Mycoplasma* mastitis. In one study from the Netherlands, 15 *M. bovis* outbreaks are described, occurring from 1976 to 1992. In all instances the infection was eradicated from the herds (36). The bad news is that although standard control recommendations exist, there is actually limited evidence to support their effectiveness and some evidence that control measures don't make any difference (20,27).

Let's start with the routine control recommendations (10,11,20). To prevent transmission, infected animals should be identified and culled (see caveat below) or segregated from the rest of the herd. Adequate monitoring of infection status may require repeated testing of all animals, regardless of clinical signs or SCC, including follow-up on heifers and dry cows at calving. Some people go by the adage "once a *Mycoplasma* cow, always a *Mycoplasma* cow" whilst others state that spontaneous and complete recovery may occur. If infected cows are maintained in the herd, they should be segregated from other cows at all times, i.e. both in the milking parlour and in the barn, because transmission is not limited to the milking process. To prevent contagious transmission during milking, full milking time hygiene practices must be implemented, including single service towels, gloves, and post milking teat disinfection. Use of premilking wash with disinfectant and backflush of units may contribute to a reduction in risk of transmission, and any faults in the functioning of the milking machine must be corrected. Interestingly, there don't appear to be any peer-reviewed experimental studies to support the efficacy of post-milking teat disinfection or particular active compounds.

Here is the frustrating part: the *Mycoplasma* research team at Washington State University looked at factors associated with time to clearance of *Mycoplasma* mastitis in 18 dairy herds. Most herds (14 of 18) cleared the problem within 1 month, which is good news and shows that *Mycoplasma* detection is not necessarily the first sign of impending disaster. Because all herds used more or less the same milking time hygiene measures (disinfection udder wash, individual cloths, disposable gloves, post dipping and backflush), there was no measurable impact of these measures on time to clearance. Scientists like to invoke "lack of statistical power" as the explanation for the absence of a measurable impact, and this may apply here too, particularly with regards to use of gloves (which appeared to be protective). What is not so great is that culling was not associated with clearance time, i.e. half of the herds that cleared the problem within a month with culling whilst an equal number of herds cleared the problem within a month without culling (27). That makes it difficult to know what to recommend to a farmer or herd manager upon initial detection of *Mycoplasma*. Monitoring may be enough to determine that the problem has solved itself, but it could equally be the way to find out that the problem has escalated. We need to be honest about this uncertainty when discussing potential herd management and culling strategies.

Environmental Sources

Despite gaps in the evidence, *Mycoplasma* spp. are routinely considered to be contagious pathogens. Possibly as a result, there is very little work on potential environmental sources or reservoirs. In Utah, *Mycoplasma* has been found in bedding sand from several dairy farms with mycoplasma-positive bulk-tank milk (16). Follow-up studies showed that *Mycoplasma* spp. survived in the sand pile for 8 months. The concentration within the sand pile was related to temperature and precipitation and with the growth of gram-negative microorganisms. The authors suggest that the latter observation may be due to biofilm formation (16). *M. bovis* in biofilm is considerably more resistant to stress, including heat and desiccation, than planktonic cells and biofilms may contribute to environmental survival (22).

Use of 0.5% sodium hypochlorite or 2% chlorhexidine were efficacious in eliminating Mycoplasma spp. from contaminated bedding sand (16). It is currently unknown whether bedding, particularly bedding contaminated with *Mycoplasma* positive milk, plays a role in transmission in UK herds.

CONCLUSIONS

Detection of *Mycoplasma* in milk samples is cause for concern but not for panic. Elimination of *Mycoplasma* from dairy herds has occurred spontaneously and after rigorous implementation of test and cull programs. Biosecurity to prevent introduction of *Mycoplasma* must be considered in all herds, particularly in those that are expanding or outsourcing heifer rearing. Within-herd biosecurity to prevent transmission of *Mycoplasma* is generally based on good parlour hygiene whilst good environmental hygiene may contribute. When *Mycoplasma* positive cows are segregated from the herd, they must not be combined with *Mycoplasma* negative cows in a hospital pen as conditions in hospital pens may contribute to outbreaks. Elimination of *Mycoplasma* from a herd may take anywhere from weeks to years and a herd-specific control programme and cost-benefit analysis will be needed.

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The Dairy Group, The University of Nottingham and DairyCo

RECYCLED MANURE SOLIDS AND GREEN BEDDING

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SUMMARY

Recycled manure solids (RMS) (often colloquially known as 'green bedding') have been used as a bedding material for a number of years in several countries. There has been recent interest in, and uptake of, this practice by dairy farmers in the UK. There are significant uncertainties with respect to the risks to animal and human health from using RMS bedding. A scoping study funded by DairyCo, revealed that published work on the subject is very limited. The majority of work on animal health implications has been in relation to mastitis pathogens. Total bacterial counts in fresh RMS of 10^4 to 10^8 cfu/g fresh bedding have been reported. However, when used bedding materials are compared, the difference between RMS and other materials is smaller than might be expected. Very little of the information on the relationship between use of RMS and clinical and subclinical mastitis in the literature is peer reviewed and findings are not consistent. When making reference to experiences in other countries, it is important to remember that climatic conditions differ between the UK and countries where RMS has a longer history of use. This is likely to have an impact on bacterial survival and proliferation and therefore risk. Further work is currently being undertaken in an attempt to fill some of the gaps in our existing knowledge.

INTRODUCTION

Recycled manure solids (RMS) (often colloquially referred to as 'green bedding') have been used as a bedding material for dairy cows for a number of years in a number of countries. The cost and availability of more conventional bedding materials has in part resulted in interest in, and uptake of, this practice in the UK. There are significant uncertainties with respect to the associated risks to animal and human health from using RMS bedding, which in turn makes it difficult to establish whether the material can meet the necessary requirements for safe use as defined under the EU animal by-products regulation (EU 1069/2009).

A scoping study was funded by DairyCo to review the current knowledge with respect to the use of RMS as bedding for dairy cattle In addition a survey of UK RMS use and bacterial load was carried out. This study revealed that the amount of published work on the subject is very limited. With respect to animal health implications, the majority of previous work has been in relation to mastitis pathogens and this is the aspect considered in this paper.

THE RISK OF RMS TO UDDER HEALTH AND MILK QUALITY

Risk pathways associated with bedding use were drawn up for animal health, human health and milk quality and are outlined in Figures 1 and 2.

Figure 1 Risk pathway for udder health

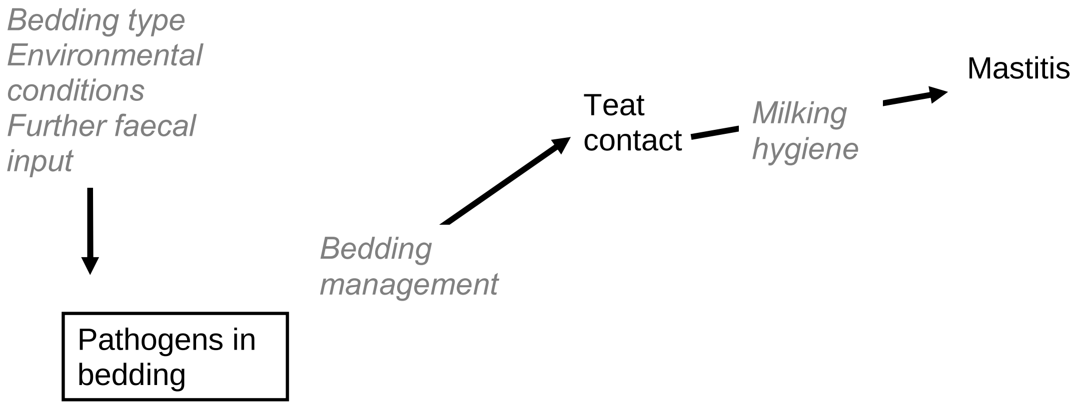
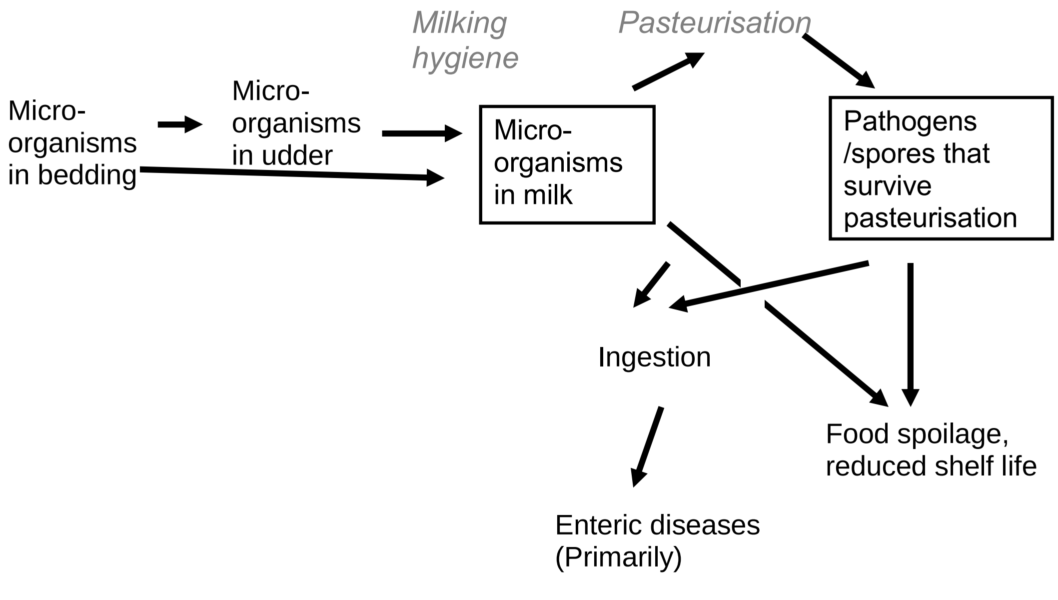


Figure 2 Risk pathways for milk quality and human health



These pathways suggest that minimising pathogen load in bedding and pre-milking hygiene are likely to be key factors in reducing any risk to udder health associated with RMS use. Whilst these are also important in protecting human health and product quality, pasteurisation will also play a crucial role here.

BACTERIAL LOAD IN BEDDING

By nature of its origin, the raw material RMS (largely the fibrous content of faeces) would be expected to contain a high load of micro-organisms and potential pathogens and this is substantiated by literature and the results of a survey carried out within the scoping study (1). The analyses of samples from UK farms compared to bacterial counts from published reports are illustrated in Figure 3.

In overview, bacterial counts appear to vary greatly both within and between different bedding materials, and the ability to compare studies is limited because of differences in the methods or units used to express results. Total bacterial counts in fresh RMS of the order of 10^4 and 10^8 cfu/g fresh bedding have been reported. Fresh sawdust shows a similar range and even "fresh" sand, claimed to be inert, can provide some samples with very high load. With use, there is a trend for all products to move towards or beyond the higher end of the range for fresh material. *Klebsiella* spp have been implicated as a particular issue with RMS bedding. However, counts are extremely variable within bedding types, and have been reported at least once at relatively high levels (10^4 cfu/g or more) in all materials both before and after use, apart from sand.

Figure 3a Total bacterial counts in different bedding materials

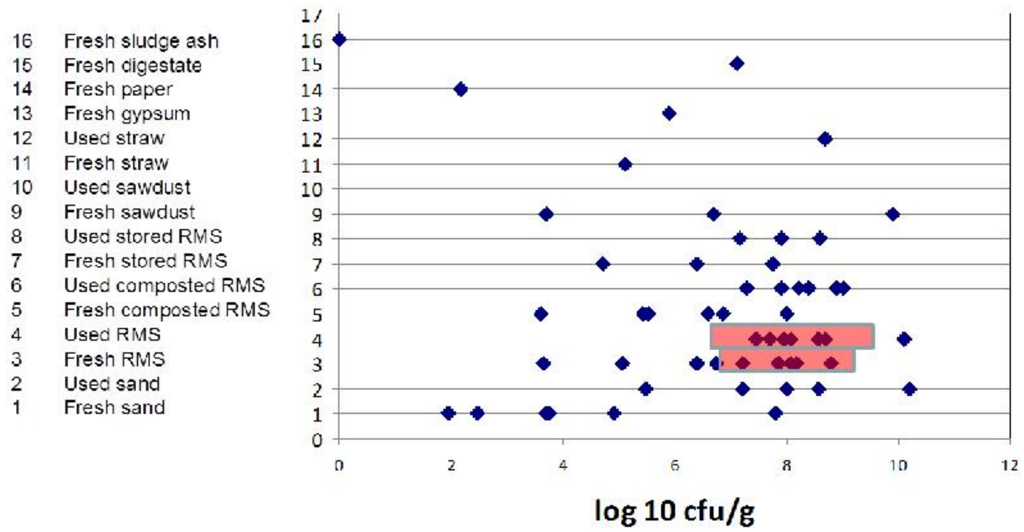


Figure 3b Coliform counts in different bedding materials

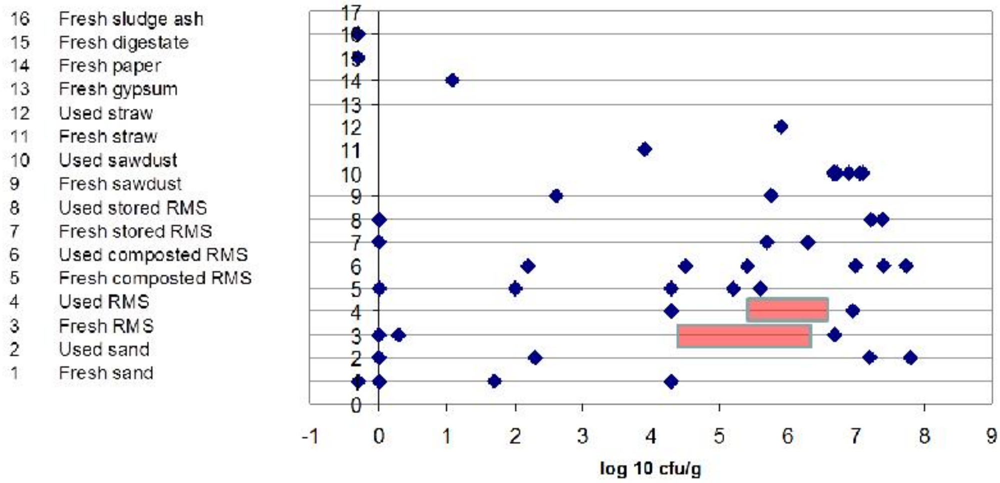


Figure 3c *Staphylococcus* spp counts in different bedding materials

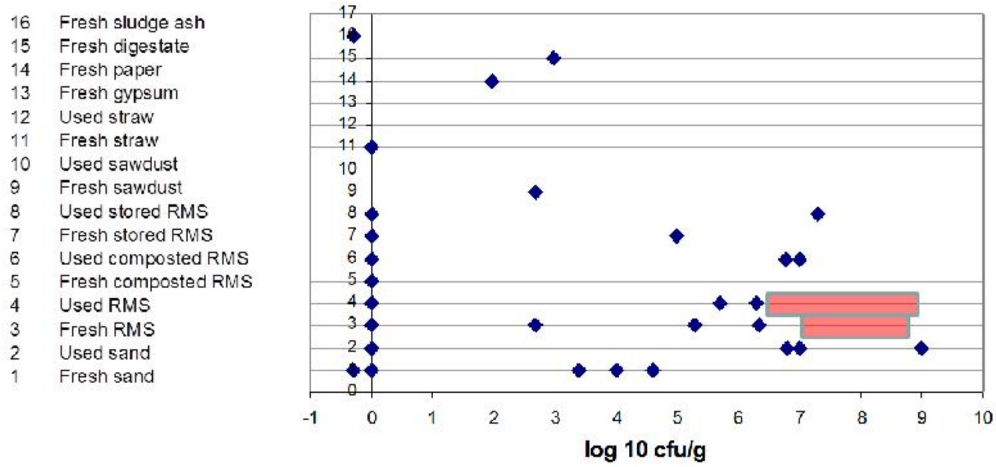
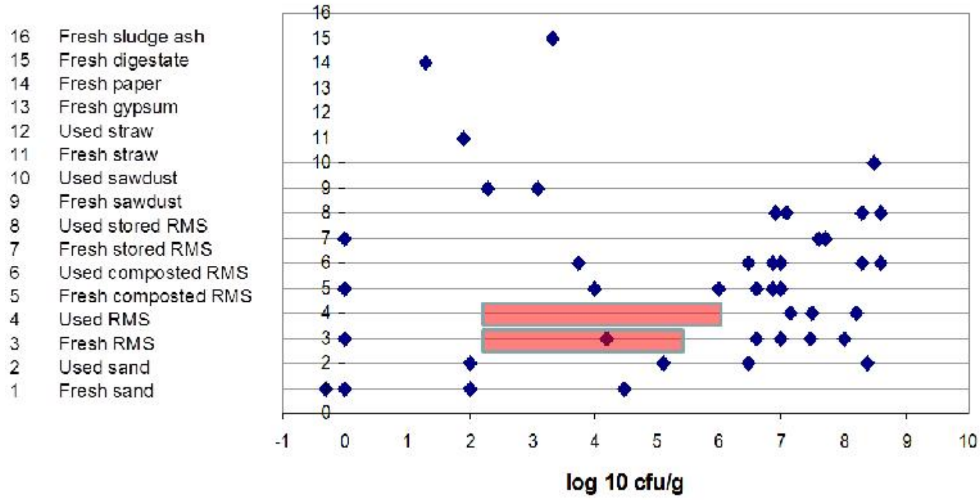


Figure 3d *Streptococcus* spp counts in different bedding materials



Footnote: Shaded boxes represent range of results from UK survey
Diamonds represent data from published reports (2,3,4,5,6,7)

THE IMPACT OF RMS ON UDDER HEALTH AND MILK QUALITY

This has generally been the greatest concern among those considering the use of RMS as bedding. Information is available on the pathogen load on bedding of various types, as outlined earlier, however, the strength of the relationship between pathogen load on bedding prior to application and intramammary infection is unclear. There are several papers that indicate that the pathogen load on the "fresh" bedding material is less important than the way the bedding is managed once applied. Rendos et al (8) demonstrated a relationship between bacterial populations in bedding and on teat ends for straw, sawdust and shavings, though Bishop et al (9) failed to do so for a comparison between composted manure solids and rubber mats. Although the manure solids had a higher bacterial load, this was reflected in the teat end swabs only for *E. coli* and *Enterobacter* spp and was not reflected in results from bacteriology of milk samples (9).

Some information on the relationship between use of RMS and clinical and subclinical mastitis is available in the literature, but very little is peer reviewed. Husfeldt & Endres (10) studied 34 herds in the US mid-west bedded on various types of RMS, including deep beds and RMS applied to mattresses. The herds showed a wide range of mastitis incidence, from 10 to 109 cases / 100 cows / year (mean 62) on deep beds, and 13 to 108 cases/100 cows/year (mean 49) on mattresses. There was no significant difference between the two types of stall surface (RMS on mattresses and in deep beds). An overview of culling revealed that the predominant culling reason in the RMS study herds was mastitis, whilst reproductive problems were the predominant reason for culling countrywide. These authors considered, on the basis of these findings, that RMS might compromise udder health in these herds, although surprisingly they based these conclusions on comparisons with mastitis rates seen in other countries rather than in the US.

Harrison et al (5) found that bacterial levels in bedding were not closely associated with the number of animals with increased SCC. Information on somatic cell counts from 38 herds using RMS in the US was summarised by Husfeldt et al (11). The average SCC of 274,000 cells/ml (+/- 98,000) was considered to compare favourably with an average of 305,000 cells/ml reported in sand-based freestalls in Minnesota (12). In a further unpublished report, Endres and Husfeldt (13) concluded that excellent cow preparation at milking time, sanitation of milking equipment, cow hygiene, adequate dry cow housing and bedding/stall management appear to be critical in maintaining a low SCC while successfully using manure solids for bedding.

As part of the scoping study, clinical mastitis and/or SCC data was collated from 10 UK farms which had started using RMS (1). A comparison of performance before and after implementation of RMS as bedding was made on this small cohort of herds. In addition, this small cohort was compared to a larger population of herds recording with QMMS Ltd. Both these comparisons failed to demonstrate any consistent effect (positive or negative) of the use of RMS as bedding. Whilst the authors acknowledge that these types of comparison are not the most robust way of measuring the impact of RMS, they presented the only possibility at the current early stage of adoption of RMS in the UK.

MITIGATING POTENTIAL RISKS TO UDDER HEALTH AND MILK QUALITY POSED BY RMS

As a result of the scoping study, 'best practice' guidelines were drawn up by the industry for the use of RMS as bedding. These guidelines have subsequently been incorporated into the Red Tractor Farm Assurance Scheme and are available on line (http://www.dairyco.org.uk/technical-information/buildings/housing/recycled-manure-solids/#.VD-qlBNwaic) accessed 16/10/14.

Key factors in maximising milk quality and minimising any risk to udder health are likely to be the same as for any other bedding material, albeit that their proper implementation may be more critical, given the higher initial bacterial load in RMS. Examples of such factors are:

- Maintaining dry beds free of contamination with fresh faeces
- Ensuring adequate ventilation
- Ensuring an adequate pre-milking teat preparation routine that includes pre-milking teat disinfection
- Pasteurisation of milk from farms using RMS to minimise the risk of transfer of zoonotic pathogens and organisms likely to impact end product quality.

Less clear at this stage is what, if any, long term implications there may be from the prolonged use of RMS. Examples of such unknowns are the impact of Johne's disease and adaptation of pathogens in terms of virulence and antimicrobial resistance, when they are continually recycled in a system which is relatively closed compared with that existing with conventional bedding materials and field disposal (and dispersal) of manure.

CONCLUSIONS

There are a number of unknown issues surrounding the use of RMS as bedding for dairy cows in the UK. Whilst some of these concerns may apparently be addressed by reference to experience in other countries, it is important to remember that the climatic conditions are different between the UK and countries where RMS has a longer history of use, and this is likely to have an impact on bacterial survival and proliferation and therefore risk. These unknowns are acknowledged by the industry and further work is currently being undertaken in an attempt to fill some of the gaps in our existing knowledge.

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EVALUATION OF TEAT COVERAGE WITH AN AUTOMATIC POST MILKING TEAT DISINFECTANT SYSTEM USING SIX DIFFERENT SPRAY DURATION SETTINGS

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INTRODUCTION

A study of manual post-milking teat disinfection using vacuum operated hand held teat sprayers in 2013 (1) identified that on average only 3.77 of teat ends were "hit" with chemical and 50% of teat barrels were covered. There was also significant variation between operators. It was suggested that an automatic system would remove this variation and apply disinfectant more consistently. In this study Locate'n'Spray™ automated teat spray devices were installed at six stalls on a 60 point rotary parlour. Between June and the beginning of August 2014 an observation and evaluation study was carried out by *The* Dairy Group over 12 milkings, using six different spray timing regimes.

The objective was to assess teat barrel and end coverage.

EVALUATION METHOD

Teat barrel and teat end coverage were assessed post application of the teat disinfectant product, using the system described in 2013 (1).

The spray duration regimes evaluated were:

➢ 0.5 seconds	➢ 1.0 second
➢ 0.75 seconds	➢ 1.0 second given in two pulses, each of 0.5 seconds with a 4 second interval (double hit)
➢ 1.5 seconds	➢ 1.5 seconds given in two pulses, each of 0.75 seconds with a 4 second interval ((double hit)

The spray duration regimes were set randomly.

The aim was to obtain teat coverage scores for each spray regime for at least 100 cows. Due to herd size of around 550 cows in milk, and 10% of stalls having the automatic spray system installed, the evaluation was carried out at the afternoon and the following morning milking. . Due to the seasonal calving pattern, the number of cows in milk for the 0.75 second spray duration was less than other regimes.

RESULTS

The study average results for teat coverage when all Locate'n'Spray™ devices were triggered are given in Table 1.

Table 1. Teat end and teat barrel coverage

		Volume of disinfectant used (ml)	Average Number - Teat end coverage	Number for No teat end coverage	Number of missing quarters	Average % for Left teats	Average % for Right teats	Average % for Rear teats	Average % for Front teats	Average % for All teats
0.5 seconds		18.4	3.84	15	1	60.55	63.13	64.48	59.15	61.83
0.75 seconds		29.6	3.87	8	0	73.63	68.43	71.05	71.01	71.03
1.0 seconds		41.6	3.89	11	0	86.54	86.59	83.23	89.90	86.57
1.5 seconds		36.8	3.92	8	0	91.05	90.58	90.60	91.03	90.81
2 x 0.5 seconds		67.2	4.00	0	0	89.58	88.24	87.48	90.35	88.91
2 x 0.75 seconds		60.8	3.94	6	0	89.35	88.44	88.22	89.57	88.89
	STUDY AVERAGE	-	3.91	8.00	0.17	81.78	80.90	80.84	81.83	81.34
	Minimum	18.4	3.84	0.00	0.00	60.55	63.13	64.48	59.15	61.83
	Maximum	67.2	4.00	15.00	1.00	91.05	90.58	90.60	91.03	90.81

The 1.0 second spray duration gave teat barrel coverage which was higher than the best coverage recorded in the 2013 manual teat spraying study, with the 0.5 seconds spray duration as good as the manual sprayer average and with similar amounts of disinfectant used.

CONCLUSION

A conclusion of the 2013 study was that "*An automatic system that applies the product consistently and achieves acceptable levels of teat barrel and teat end coverage would be advantageous to the industry*". From the results of this spray duration study the Locate'n'Spray™ system achieves this aim, with a minimum of 96% of teat ends covered with disinfectant and between 61.8% and 90.8% of teat barrels covered (depending on spray duration).

Therefore the automation of Locate'n'Spray™ provides a level of process control which delivers consistently superior teat coverage and consistently higher teat end hit rates compared with manual spraying. The associated benefit of time saving in the parlour allows better targeting of labour, benefiting udder health and milking management, but partly offset by higher chemical consumption.

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BEST PRACTICES TO PREVENT MEDICINE RESIDUES IN MILK

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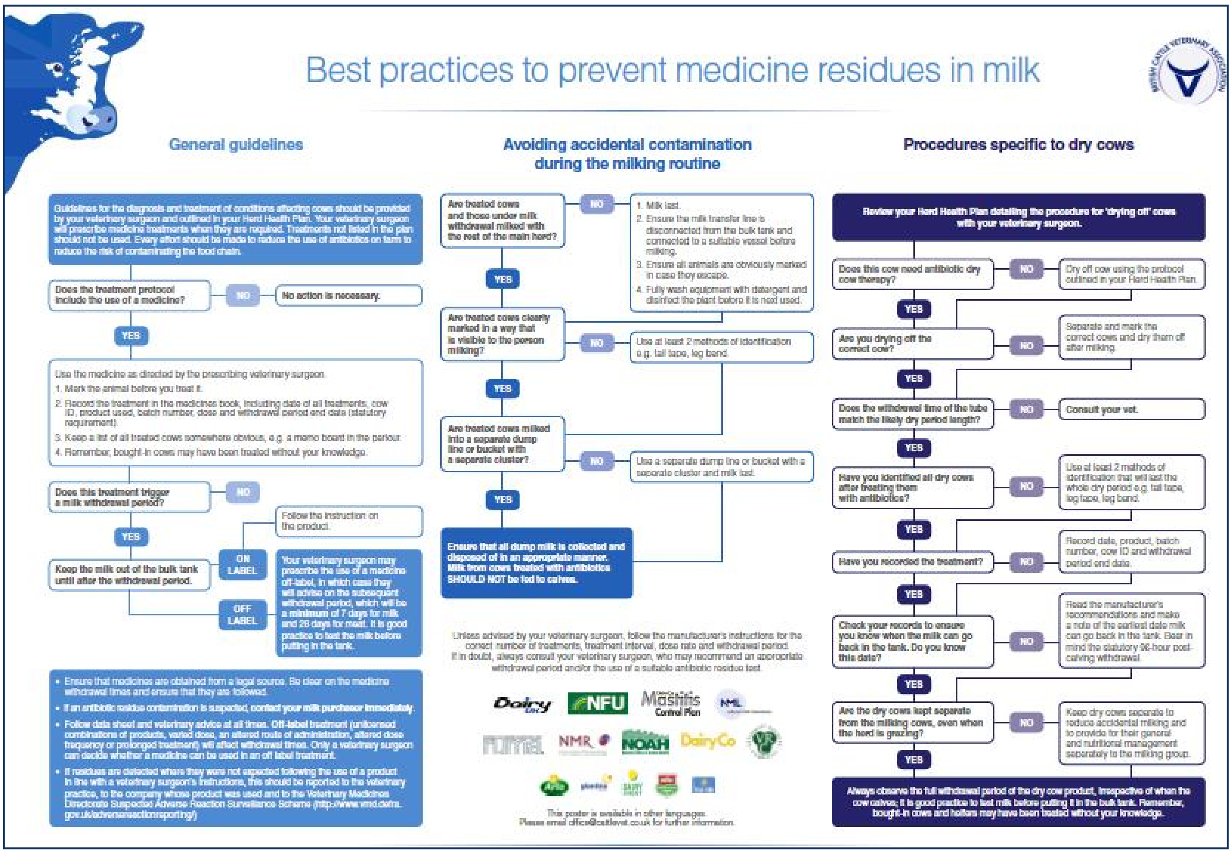
SUMMARY

BCVA in association with NOAH, VRC, RUMA, DairyCo, NFU, NMR, NML and DairyUK have produced and distributed a poster guide for milk producers entitled “Best practices for preventing medicine residues in milk”. The poster is an updated version of the practical guide to avoiding milk antibiotic residues that was last produced in 2007.

The poster has been distributed to all registered dairy producers in the UK throughout March and April 2014. It was preceded or accompanied by a postcard encouraging producers to “display the poster in a location that allows it to be used as a source of reference when treating milking cows with medicines”.



The poster has been translated into:

- Welsh
- Polish
- Estonian
- Latvian

The large poster (A2) in English is available to purchase from the BCVA Office whilst translated versions of the large poster can be pre-ordered from the BCVA Office: office@cattlevet.co.uk.

All posters are available in electronic pdf format to download from the BCVA website www.bcva.org.uk/http://www.bcva.eu/bcva/news/medicine-residues-milk-guidance-poster . Translated versions of the large poster can be pre-ordered from the BCVA Office: office@cattlevet.co.uk.

We welcome your support in ensuring all farms display this poster as a reference to preventing medicine residues in milk.

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SELECTIVE DRY COW THERAPY USING ORBESEAL ™

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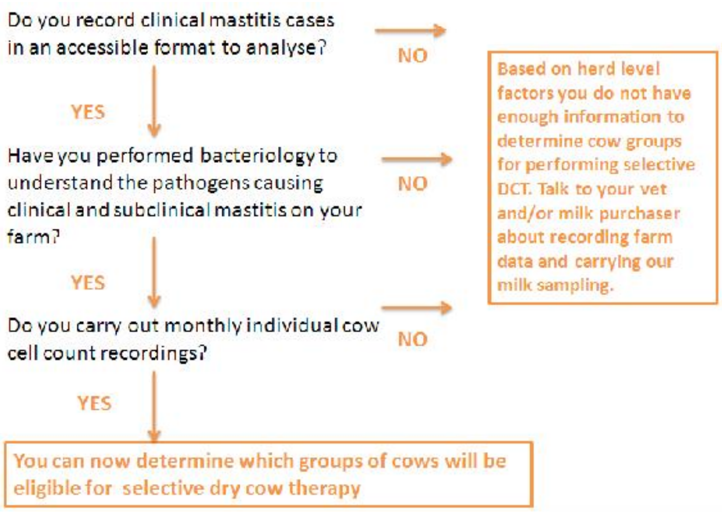
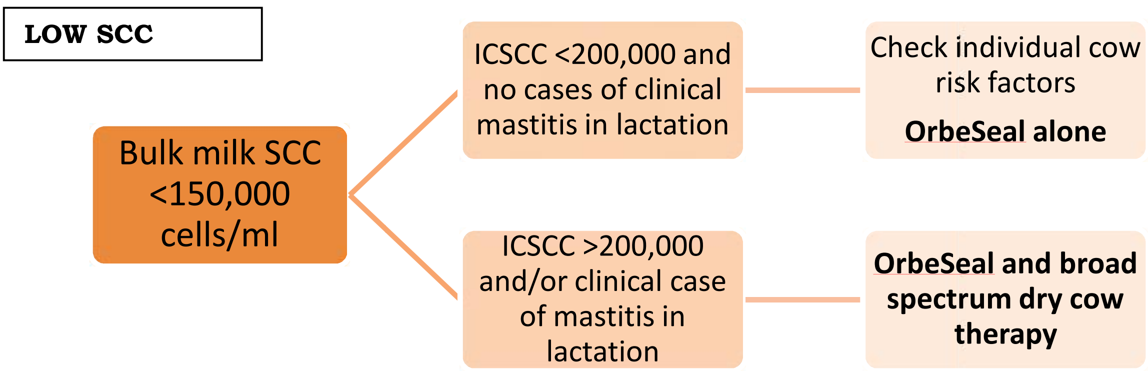
SUMMARY

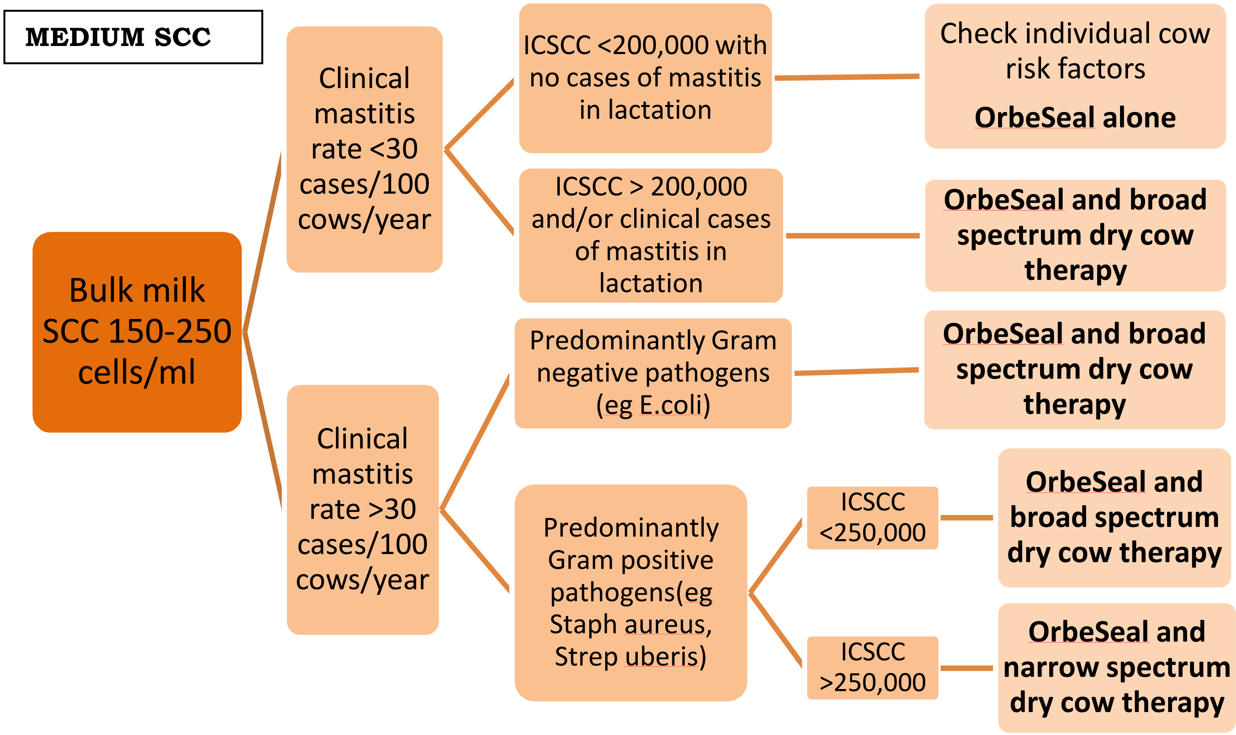
Selective dry cow therapy is increasingly becoming important in UK dairy herds. The decision about whether a cow should receive antibiotic dry cow therapy (ADCT) in addition to an internal teat sealant or an internal teat sealant alone should be based on farm information including somatic cell count recordings, clinical mastitis case rate and knowledge of the predominant mastitis pathogens on the farm. At Zoetis we believe it is important that selective dry cow therapy using OrbeSeal is performed correctly using all the farm information available. The flowchart below is designed to offer a guide to decision making for determining the appropriate dry cow therapy at the herd, group and then individual animal level.

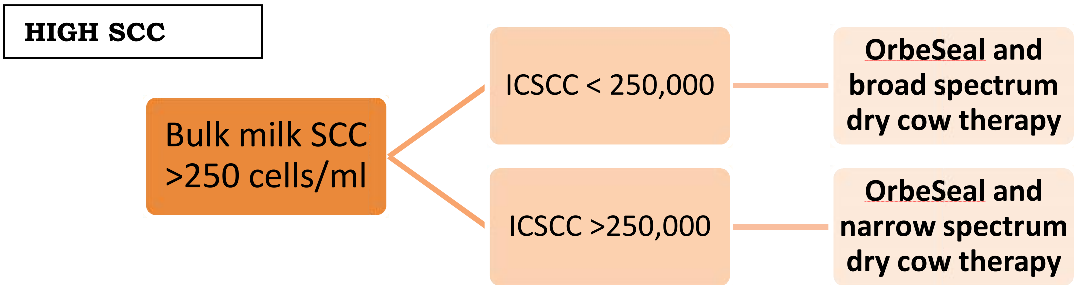
Stage 1 – Herd level



Stage 2 – Group level







Individual cow risk factors for mastitis

- ***Environment***: high bacterial populations at the teat end
- ***Production***: high milk production across lactation, poor or delayed formation of keratin plug
- ***Drying off***: increasing parity, high milk production at drying off, gradual method of dry off, poor hygiene and cleanliness at dry off, milk leakage at dry off, poor teat end integrity (eg damage, hyperkeratosis)

CONCLUSIONS

Selective dry cow therapy is important in UK dairy herds to reduce the usage of antibiotics and on some farms as part of a mastitis control programme. OrbeSeal prevents mastitis acquired during the dry period and is therefore a vital tool in mastitis control, whether used with or without an antibiotic dry cow product. The use of OrbeSeal alone should be targeted at cows which have a reduced risk of having a mastitis infection at dry off. OrbeSeal should always be infused in a clean and hygienic manner to reduce the risk of iatrogenic infection.

To discuss the use of OrbeSeal either alone or with an antibiotic dry cow product further, please contact your veterinary surgeon or a member of the Zoetis Ruminant Technical team or email: customersupportUK@zoetis.com

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USING MEDICINES AUDITS TO MONITOR AND DRIVE RESPONSIBLE ANTIMICROBIAL USE IN FARM ANIMAL VETERINARY PRACTICE

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All antimicrobials licensed for use in food producing animals in the UK are prescription only medicines that may only be given following a clinical assessment of the animal or group of animals, diagnosis and prescription by a veterinary surgeon (POM-V). Hence, even though they may not be directly administering the medicines, ensuring responsible use on farm is an essential part of a veterinary surgeon's role (1,2).

Protecting animal health, human health and the food chain at source through zoonotic disease surveillance and prevention of medicines residues - particularly antimicrobials - is consistent with the One-Health approach (3). A maximum residue limit (MRL) is determined for all POM-V medicines which dictates the withdrawal period after administration during which meat, milk or eggs may not enter the food chain. The prescribing veterinary surgeon must make sure that farmers are aware of and adhere to these. Confronting partial compliance with treatment protocols is a common challenge (4).

There is increasing public and scientific concern about the potential transfer of antimicrobial resistance (AMR) from food-producing animals to humans (5), although the main driver of AMR in people is use in human medicine (6). Current veterinary prescribing practices are under review (7, 8), and a mandate for and guidelines on responsible use have been produced by the Royal College of Veterinary Surgeons (9) and BVA (2), respectively.

In particular, the use of certain "protected antimicrobials" (quinolones, 3rd and 4th generation cephalosporins and long-acting macrolides) in food- producing animals, because of their importance in treating resistant organisms in humans, is being challenged. However, certain examples present a paradox because they do not partition into milk above the MRL, and so are potentially useful in helping to prevent antimicrobial residues.

Farm animal veterinary work is true population medicine, where the emphasis is on monitoring, managing and preventing disease in groups of farm animals, rather than focussing on solely treating individual cases. This approach has been well summed up by the concept of "herd health management" (HHM). Reducing total antimicrobial use while driving more responsible use on farms, referred to as optimising antimicrobial use by the Department of Health (6), is likely to have the biggest impact in slowing AMR development, but this can only be achieved by improving management systems so that animal health and welfare are not compromised. Integrating medicines audits with HHM provides the vehicle through which this change can be achieved.

In an effort to reduce antimicrobial use and drive more responsible use, both farm and practice-level medicines audits were adopted as part of HHM and clinical governance, respectively, by Langford Farm Animal Practice, a clinical teaching practice of the University of Bristol, School of Veterinary Sciences. Practice prescribing policy was changed to reduce the reliance on "protected antimicrobials". Farmers' meetings and regular newsletter articles were used to raise awareness, provide training on compliance and responsible use, and to encourage engagement. Practice veterinary surgeons engaged proactively with farm clients as part of ongoing HHM in order to reduce total antimicrobial use. An Excel (Microsoft Office, 2010) spreadsheet was developed to capture and analyse the data collated from practice medicines sales, and results fed into HHM discussions as appropriate. Areas of good practice and responsible medicines use were identified, emphasised and encouraged, and poor compliance highlighted. No deterioration in herd health was observed as a result of altered prescribing practices.

Medicines audits are useful tools to both monitor and drive responsible antimicrobial use when combined with ongoing HHM. Responding to the threat of AMR, prescribing practices can be changed successfully, whilst keeping farmers on board and without detrimental effects to animal health.

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MUST KNOW FACTS ABOUT TEAT DISINFECTANTS AND THEIR ROLE IN THE PREVENTION OF MASTITIS IN MODERN DAIRY HERDS

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INTRODUCTION

Recently, Hemling (2014) noted that 'teat disinfection is the most important second in the milk harvesting process, in terms of milk quality, mastitis control, and cow longevity'. This second refers to the time that it takes a milker to dip a teat in a disinfecting solution during the milking routine, with the major objective of eliminating microorganisms that could compromise the health status of the mammary gland. The investment in time and money in this critical step of the milking process could mean a world of a difference for the farmer's pocket when considering the high economical costs and the impact on cow longevity of a single mastitis case. This paper aims to summarize and clarify the basic concepts about teat disinfectants components, and provide guidelines for the practical use by the dairy producer.

THE COST OF MASTITIS AND OF TEAT DISINFECTANTS INTO PERSPECTIVE

There is little doubt that the cost of a clinical mastitis case is hurtful for the dairy farmer's pocket. Kossaibati & Esselmont (2000) calculated the cost to be £80.35 per mild clinical mastitis case (where the herdsman applied the treatment) or £207.97 per severe case (where veterinarian intervention was needed). Milk loss, in terms of discarded milk and reduced milk yield make up the majority of the cost per clinical case. For a mild case, milk loss totalled 327 litres (85% total cost), while for severe cases it was 570 litres (57% total cost). In Ireland, recent data by Geary *et al.* (2012) estimated the milk yield loss at 177-601 litres, depending on the bulk tank cell category of the farm. In the USA, Bar *et al.* (2008) also calculated the generic costs of a clinical case of mastitis in freestall production systems.

Managing the cost of teat disinfectants and other variable costs is a constant worry for the farmer, who aims to maximize the profit per litre of milk produced. Comparatively, the total for these variable costs is about £1.55/d while that of feeding is £2.62/d for a cow producing 23 litres of milk per day (Old Mill, 2013). The estimated cost of post-milking teat disinfection for a cow is ~£0.08/d, which is ~5% of the variable costs and only 1% of the total production costs. Seen in another perspective, the value of dipping a cow for 305 days is about quarter of the cost associated with the milk losses of a mild case of mastitis (327 litres with a milk price of £0.2991 = £97.81). When used properly in the milking routine, disinfecting cow teats will have a beneficial outcome on cow health and farm economics.

In addition to post milking teat disinfection, recommended management strategies to prevent mastitis include good hygiene at milking time, routine maintenance of the milking machine, adequate type of bedding and bedding material, and balanced nutrition. Specifically in the milking routine, the National Mastitis Council (NMC) recommends forestripping quarters before milking, the use of teat disinfectants before and after milking, and the use of single paper towels to clean and dry teats. Considering these prevention practices, the reduction in the rate of new intramammary infections (NIMI) is generally >50%, depending on the type of pathogen, whether it is contagious or environmental. A model developed by Allore & Erb (1998) evaluated the economical benefit of implementing these prevention strategies, plus the use of lactational therapy (LT) and dry cow therapy (DCT) for managing contagious or environmental-dominant infections on farm. They concluded that for contagious pathogens farms, prevention practices and DCT had the most beneficial economical effect. For environmental pathogens farms, prevention practices, LT and DCT and vaccination (i.e. for *E.* coli) proved to be the most beneficial. Whatever type of mastitis pathogens are found in the farm, teat disinfectants are the cornerstone for management of the disease and will become increasingly important as antibiotic use will be more restricted. Dairy farms that judiciously integrate postmilking teat disinfection into their milking routine are successful at maintaining low clinical mastitis rates. Ruegg (2004) observed a 50% difference ($P<0.01$) in monthly clinical mastitis rates between farms that practiced a full milking routine vs. those that did not.

WHAT IS IN A TEAT DIP?

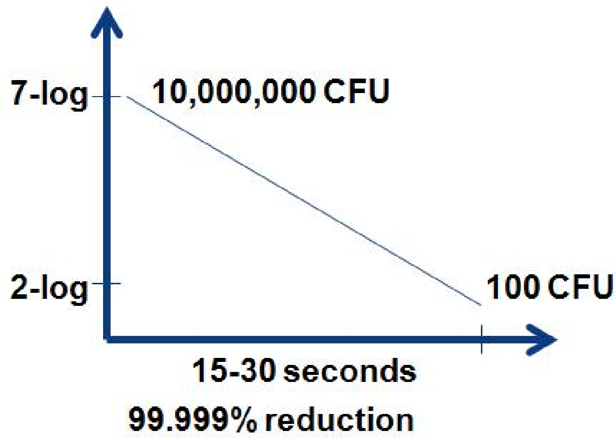
The making of a good cocktail requires knowing its main components and their function. In a similar manner, knowing the most important components, and the roles that they play in the formula are important for choosing the best teat disinfectant for your needs. In the case of teat disinfectants, the four major components are: germicide, emollients, surfactants, and water. The proportion of these, added to other minor components (e.g. dye, film formers, thickeners, among others) are formulated to match the function of their use. Hemling *et al.* (2011) summarized the main characteristics, benefits and risks of different types of post milking teat disinfectant formulations that are marketed today.

The germicide is the most important component of a teat disinfectant, and the main reason why teat dips are manufactured and used on farm. Its objective is to eliminate microorganisms that can cause mastitis, and which are present in the teat skin at the time of use. There are multiple options for the producer to choose from, but in general terms, these can be categorized into two major groups, oxidative and non oxidative. Oxidative germicides eliminate microorganisms by means of a chemical reaction. This means that the germicide will attack microorganisms in many sites, thereby ‘burning’ (oxidating) contact points. Since they are not specific in the points of contact, the probability that bacteria create resistance to the compound is minimal. The most common oxidative germicides include iodine, chlorine dioxide, hydrogen peroxide, and sodium hypochlorite. On the other hand, non-oxidative germicides include lactic acid and chlorhexidine, among others. These types of germicides attack microrganisms in specific sites, by means of physical interaction, creating rupture of membranes or interrupting enzymatic reactions. Due to the fact that their mode of action is specific, resistance to the germicide may occur. To overcome these limitations, formulations with these germicides are normally complemented with other chemicals that enhance their germicide activity. Whether the teat disinfectants are oxidative or non-oxidative, they are formulated taking into consideration the germicide used. The pH of the formula is normally a feature that is important for the germicide, in a manner that its efficacy is nor hindered, which is why some teat disinfectants may be more acid than others. Nevertheless, patented technology in the chemistry of the formula has been used to overcome these challenges, and for instance can be associated with a formula with a pH that is more mild to the skin, without adversely affecting its germicidal efficacy (Foret *et al.*, 2004).

The efficacy of the product refers to the capacity of the product to kill microorganisms, and thereby help prevent mastitis from occurring. Again, this is the major reason why dairy farmers purchase a teat disinfectant. Proof that a teat disinfectant meets both objectives can be proved by lab and field studies. Lab studies are normally standard and accepted tests that will quantify the reduction of bacteria in a determined period of time. These tests will require that a disinfectant reduce a bacterial population by at least 5-log (99.999% reduction) in a determined time to pass the test (examples EN 1040, EN 1656). Although the standard tests require testing the disinfectant against a limited number of bacterial pathogens, modifications to the test including the testing of additional pathogens that are mastitis specific, or testing at reduced times, are ways to demonstrate more farmer practical data (Lanckriet *et al.*, 2014). For practical on-farm applications, for example, any teat disinfectant that is used before milking should be able to kill bacteria present on teat skin in 30 seconds or less, which is the average time that a disinfectant is left on teats before removal (Figure 1). Post milking teat disinfectants need to kill bacteria present at the teat orifice before it gets pulled into the udder

Field efficacy studies are aimed at demonstrating that a teat disinfectant can effectively help reduce NIMI over a determined period of time. Protocols for running these trials are available at www.nmc.org. However, modifications to these protocols considering novel statistical methods and sampling procedures have been proposed (Schukken *et al.*, 2013, Ceballos-Márquez *et al.*, 2013). Many years ago these studies were conducted comparing cows that were disinfected vs. those that were not (negative control). Results of studies showed that a minimum of 40% reduction in NIMI was to be expected when using a teat disinfectant vs. a negative control (Schukken *et al.*, 2013). Because of animal welfare concerns it is very difficult to run these negative control trials, as dairy farmers are hesitant to not use teat disinfectants and put their cows at high risk of infection. In university studies, animal ethics committees have also raised the issue on whether cows should be left without teat disinfection, thereby risking their welfare over the duration of a trial. This is why in studies conducted over the last few years require that a test product be compared to an existing teat disinfectant (positive control) that is commercially available. A successful trial should be able to demonstrate that a test product is as efficacious as the control product in terms of rate of NIMI over the duration of the trial period.

Figure 1. Schematic diagram of a successful lab study aimed at demonstrating the efficacy of a teat disinfectant. (Source: author)



The second main component in a teat disinfectant formula is the emollient. The interaction between the animal and the milking machine should allow a smooth milk harvesting procedure. Because the teats are manipulated constantly throughout the day, by chemical or physical means, maintaining optimum teat condition health is very important. If the teat is dry, for example, squaks and liner slippage may occur. If the teat skin is cracked, milking can cause pain to the cow and may affect milk yield (McKenzie & Hemling, 1995). Neijenhuis *et al.* (2001) showed that there is a positive correlation between high teat end scores with presence of mastitis in herds. The most common teat condition problems, their cause and solution were summarized by Ohnstad *et al.* (2007). Teat condition scoring of the herd should be monitored on a regular basis to ascertain whether the emollients present in the teat disinfectant are meeting their purpose. Scoring is relatively easy and can be easy implemented as part of a monthly evaluation by a milk quality specialist or by the dairy producer.

Humectants such as glycerin and sorbitol are the most common materials used as emollients in teat disinfectants. When they are applied topically, they draw water from the dermis to the epidermis, but very little from the environment (Draelos, 2000). Pre and post-milking teat disinfectants generally have between 2-10% emollients while winter-type disinfectants may have emollient levels between 25-50%. It is important to highlight that the emollient level alone is not a guarantee of improving teat condition. In the formula there may be components that irritate the skin, which may not be solved by the additional increase in emollients alone. At the end of the day, it is the complete formulation, germicide, pH, surfactant type and concentration, and emollient that dictate the teat condition outcome.

The third component in the great majority of teat dips are surfactants. These components tend to play various roles within the formulation, such as detergent (helps to remove soil from the skin), solubilizing agent (complexing agent in iodophores), foaming agent, or emulsifying agent (Mishra *et al.*, 2009). For cleaning purposes, for example, by helping to reduce superficial tension between the liquid solution and the skin, better penetration of the product in skin is achieved. The levels of inclusion in a formula vary according to the expected function. For instance, a pre-milking teat disinfectant may have a higher quantity of surfactants that enhances teat cleaning during the milking prep routine.

The fourth component in any teat disinfectant formulation is water. Its proportion within the formula varies on whether the product is formulated as a concentrate or a as a ready to use solution. When concentrates are used, it is important to know the type of water that is available on farm for use in the dilution process. Although water quality used in the manufacturing process can (and should) be guaranteed by the manufacturer, the water quality at the farm level is more uncertain and could be different to manufacturer's label recommendations (if any). It is recommended that a dairy producer understand the physical and microbiological characteristics of water available on farm if there is a need to use it to dilute concentrates (NMC, 2009).

There are other components that are normally added to teat disinfectants, and these give the formula special features that make them available for use in different applications. Some components may be used to increase the viscosity of the product so as to reduce dripping. Others help to create flexible films that can act as physical barriers to soiling and contaminating agents. Not last, but not least, dyes are normally added to formulas because they help farmers understand when a cow has been dipped or not, for example.

Teat disinfectants are a basic component of a good milking routine. They are manufactured taking into consideration their germicidal activity and their functionality within the milking routine. They are also formulated with special consideration to the needs and preferences of the market, regarding their germicides or physical properties. Many decades of use worldwide and proven results on farm have demonstrated that the small amount of time dedicated to dipping a teat can have beneficial results in prevention of mastitis.

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THE ROLE OF VACCINATION IN MASTITIS CONTROL

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SUMMARY

There is a role for vaccination in mastitis control based on the ongoing high economic cost of mastitis in the dairy industry, alongside a growing recognition by government and consumers that a reliance on reactive antimicrobial solutions risks unacceptable food residues or a potential risk of antimicrobial resistance impact on human health. A range of defence mechanisms are employed by the cow to resist intramammary infections and immune mediated responses are largely based on the innate immune system. Vaccination technologies are emerging from research to enter into the commercial market in the UK and a vaccine that combines antigen based on the J5 component of *Escherichia coli* (E.*coli*) with a staphylococcal biofilm antigen is now marketed as STARTVAC® (Hipra). There are challenges in delivering any vaccine programme on farm and emerging pathogens such as prototheca require a genuinely balanced herd health approach bespoke to each farm going forwards. This approach is supported by such tools as the DairyCo Mastitis Control Plan and opportunities are emerging for developing the next steps in mastitis control.

THE NEED FOR VACCINATION

Economics

Mastitis is widely recognised as the most costly disease of dairy production (6), although the magnitude of these losses varies enormously between farms. A bespoke approach to understanding these costs should therefore be undertaken (17, 24, 2). Mastitis remains an economic priority for dairy production and consequently a full range of control options is sought.

Reliance on antimicrobials

Culturally, reactive therapeutic solutions and prophylactic antimicrobial dry cow therapy remain a significant part of mastitis control. Mastitis therapy accounts for the largest proportion of antibiotic drug use in the dairy industry (20). However, despite a range of products, treatment success can be disappointing and reducing the need for antimicrobials requires investment in new technologies (38). Consumer concerns over potential residues in milk are raising expectations of the role of the practitioner in responsible use of antimicrobials and avoiding residues (3).

Antimicrobial resistance

The 'one health agenda' is driving the global need to reduce the reliance on antimicrobials in food production (14). Both concerns over antimicrobial resistance (AMR) as well as the awareness of potential residues in milk are rising in prominence (40). A survey of the prevalence of antibiotic resistance among bacterial pathogens isolated from cattle in different European countries for the period 2002 to 2004 was carried out (19). This study found that for *Staphylococcus aureus (S. aureus)* detected from bovine mastitis, major differences were apparent in the occurrence of resistance between countries and between the different antibiotic agents tested. The highest frequency of resistance was observed for penicillin. Although there remains much evidence for AMR arising in the main from within the human health community (40), the expectation of responsible use of antimicrobials in livestock food production is high (15).

The dairy industry has come under considerable criticism regarding AMR and so strategies such as selective dry cow therapy are emerging as a method to address the urgent need to justify use of antimicrobials when necessary, with appropriate economic considerations (23). Vaccination offers a contribution to the ongoing attempts to manage a costly industry issue and also reduce the need for antimicrobials and hence perceived AMR risk.

DEFENCES AGAINST MASTITIS

Mastitis is an inflammation of the udder caused by a range of invading pathogens. Clinical signs vary according to both host and pathogen specific factors. The development of effective vaccines to combat mastitis in cattle remains challenging, as despite a growing body of literature we still lack key knowledge of the host-pathogen interaction in the bovine udder (27).

Innate v Adaptive Immunity and Memory

Clinical and sub-clinical signs of mastitis are predominantly associated with the inflammatory response to bacterial challenge to the udder. Physical defences such as keratin offer the first line of defence against invading pathogens, however once these have been overcome the innate immune system offers the next level of defence (12). Immune memory and therefore adaptive immunity plays a less significant role in mastitis than in many other disease processes and so the cow relies on innate immunity, which essentially has no memory (36). Barriers between the udder and general circulation as well as between quarters may contribute to impaired mechanisms of acquired immunity. Memory T cell migration from peripheral circulation to peripheral tissues such as skin and lungs is limited (25). Previously infected cows with memory T cells present therefore may still be unable to prevent or clear cases of *S. aureus* mastitis due to inadequate expression of key surface receptors such as CD45RO (42), as this impairs effective T cell migration

Pattern recognition receptors (PRRs), also known as Toll-like receptors (TLR), found on macrophages in the udder, including toll like receptor 4 (TLR4) and CD14 recognise specific areas on ligands or 'pathogen-pathogen-associated molecular patterns' (PAMPS). Only a few of these sentinel macrophages are likely to be present and mastitis is likely to be characterised by a massive infiltration of neutrophils. Lipopolysaccharide (LPS) fragments from gram negative bacteria such as *E.coli* are recognised by TLR4 on the macrophages, not the epithelial cells, and trigger release of the cytokine tumour necrosis factor alpha (TNFalpha). TLR2 recognises lipoteichoic acid in gram positive bacteria. All TLRs eventually activate the 'nuclear factor kappa-light-chain-enhancer of activated B cells' (NF-κβ) system. NF-κβ controls DNA transcription and results in the release of additional interleukins and cyclo-oxygenases which in turn trigger an inflammatory response and neutrophil recruitment. However, immune response cytokine profiles appear to be pathogen specific; the *S.aureus* cytokine profile differs from the E.coli response. Although both *E.coli* and *S.aureus* activate TLR, *S.aureus* does not activate NF-κβ in mammary epithelial cells, ultimately leading to less inflammation and reduced pathogen clearance (36).

VACCINATION AND KEY MAJOR PATHOGENS

Coliform mastitis and J5

Coliform mastitis generates considerable losses, especially in dairy herds with a high health status and low somatic cell count where contagious pathogens are well controlled. *Escherichia coli (E.coli)* and most Gram-negative bacteria, have a characteristic and essential macromolecule in their external cell membrane called lipopolysaccharide (LPS). This LPS is the major factor of pathogenicity of the bacterium. It triggers the typical set of symptoms of hyperacute coliform mastitis. The experimental intramammary injection of LPS in healthy animals causes the same symptoms, being dose dependent, with potential death of the animal at high doses.

The functional ability of the immune system of the cow is a key factor to limit the rapid spread of *E. coli* in the udder and reduce the toxic action of LPS.

Neutrophils are key players in the fight against intramammary infections (IMI). They are responsible for sequestering, killing and eliminating the pathogen, aided by opsonising antibodies, mainly IgG2 and pro-inflammatory cytokines, which are responsible for the massive influx of neutrophils from the blood capillaries of the udder into the cistern. The rapid mobilization of neutrophils into the udder is essential in reducing the impact of clinical symptoms. (41).

The economic loss is due to treatment costs, lost quarters, and, most importantly, discarded milk and early replacement of cows. *E. coli* and other coliforms are widespread in the dairy environment and considered as the most important cause of environmental mastitis associated with clinical mastitis. It usually appears on farms in which practices for controlling contagious mastitis exist, but which are not effective in controlling the clinical disease caused by environmental pathogens.

Vaccination against coliform mastitis is a commonly implemented control strategy on dairy farms in the United States (between 40-65% of the farms apply vaccination). The most widely used vaccines are based on the J5 strain of *E. coli*. This strain is a mutant that lacks the O polysaccharide chain of the LPS, leaving the LPS antigen core exposed to the immune system (16). Unlike the O-polysaccharide chain, the composition and structure of the antigen core, is highly conserved among the various Gram negative bacteria, so vaccines with the J5 induce opsonising "anti-core" antibodies will have cross immunity against different strains of *E. coli* and other Gram-negative bacteria. The efficacy of vaccination in protecting against acute coliform mastitis has been demonstrated in several field studies. In many references, it appears that immunization with J5 does not prevent coliformIMI, but is associated with reduced severity (34).

Immunization of dairy cattle with *E. coli* J5 bacterins during the dry period has been shown to reduce the occurrence of clinical mastitis under pre-clinical conditions and field conditions (22). J5 vaccination may not be significantly associated with reduced incidence of clinical mastitis, but has been shown to reduce severity of clinical mastitis and associated production loss (41). In common with many bacterial vaccines, immunity wanes quickly and hence repeated vaccination is required to maintain efficacy. Immune responses to J5 bacterins may be improved by use of novel immunomodulators, such as saponin in oil (43). The use of a J5 vaccination program is just one part of an overall preventive programme, which includes appropriate management of risk factors (34).

Vaccination potentially has an important role to play in clinical coliform mastitis control in some herds. A UK field study (5) has investigated the use of the polyvalent Staphylococcus and coliform vaccine Startvac (Hipra SA). This study failed to demonstrate any reduction in the rate of clinical mastitis, but mastitis severity was significantly reduced with label use of the vaccine. Increased productivity as milk, fat and protein in the first 120 days of lactation was also demonstrated, mitigating the cost of intervention alongside an impact on culling and mastitis associated wastage.

Staphylococci and biofilms

Recurrent mastitis infections may often be attributed to biofilm growth of bacteria, where antimicrobial therapies are ineffective and may lead to sub-clinical chronic mastitis with elevation of somatic cell counts (SCC); (29). *S.aureus* represents a major agent of contagious bovine mastitis and its ability to form biofilm suggests that it is a possible important virulence factor in the establishment of staphylococcal infection (10).

Biofilms are a structured arrangement of bacterial cells enclosed in a self-produced polymeric matrix, adhered to an inert or living surface (10). This can constitute a protected evolutionary niche that allows bacteria to grow and survive in a hostile environment, particularly in environments characterized by a continuous fluid flow. When biofilms are formed in low shear environments, they are generally more sensitive to mechanical breakage. In addition to protection against physical and chemical environmental agents, the biofilm promotes extracellular catabolism and the concentration of nutrients on cell surface.

Biofilm is formed in three stages:

i. Cells attach to a surface, facilitated by cell wall associated adhesins, which are products of various genes. Matrix proteins including fibronectin, and fibrinogen support initial attachment of cells to a surface, the primary cell aggregates produce exopolysaccharides to facilitate clumping.
ii. Cell multiplication and formation of a mature structure consisting of many layers of cells occurs, layers are connected to each other by extracellular polysaccharides
iii. Maturation occurs, many staphylococci generate a glycocalyx, a slime layer that further protects the biofilm bacteria. The chemical nature of these slime layers is still not entirely elucidated, but evidence suggests that it consists predominantly of hydrated polysaccharides (8).

Biofilm exists as a dynamic equilibrium and when it reaches a critical mass the outermost cell layer begins to shed planktonic organisms. These bacteria are free to escape the biofilm and to colonize other surfaces (8); associated with a decrease in rate of cell division.

Production of biofilm allows bacteria to resist to antibiotic therapy, ensures infection persistence and the resistance to host immunity. Resistance to antimicrobial agents (e.g. antibiotics) of bacteria within biofilm seems to be related to several factors: a) increased difficulty of the antibiotic to penetrate through the extracellular matrix, b) reduction in cell division affecting action of β-lactam antibiotics.

Protection from *S. aureus* mastitis was associated with specific antibody production using biofilm-embedded bacteria (31). Target antigen included poly-N-acetyl β-1,6 glucosamine (PNAG) or Slime Associated Antigenic Complex (SAAC). The role of PNAG and SAAC-specific antibody production in protection from *S. aureus* bovine mastitis has been investigated. The protection level was related to the features of the immunizing strain (eg degree of biofilm formation and PNAG production and consequently to the rate of antibodies to *S. aureus* PNAG); whereas it was independent of the adjuvant and capsular polysaccharide type of the challenge strain (31). Cows immunized with a greater amount of SAAC associated with the *S. aureus* bacterin triggered the highest SAAC-specific antibody levels in serum after vaccination. SAAC embedded in a *S. aureus* bacterin of a strong biofilm-producing strain was proposed as an effective target for vaccination (32).A reduction in prevalence of *S.aureus* and a reduction in the proportion of 'first infections' (high first cell count after parturition)in the three herds studied (4).

One of the benefits of using PNAG or SAAC, as the antigenic component of the vaccine, is that no differences in serotypes have been highlighted between staphylococci spp in relation to the production of the two fractions mentioned above. Therefore, the antibodies induced by vaccination with these antigens give cross-protection against several strains of *S. aureus*.

Streptococcus uberis

Streptococcus uberis (*S. uberis*) enters the mammary gland through the teat canal, resists neutrophil bactericidal action and replicates inducing an inflammatory response. The detail of the host-pathogen interaction regarding *Strep. uberis* is complex and far from fully understood (26). Contrasting virulence is well recognised in differing strains of *S. uberis* and clonal typing technologies offer opportunities for more complete epidemiological understanding of herd infection characteristics (45). In contrast to immunological responses described for *S. aureus*, following *S.uberis* infection, various cytokines (such as IL-1β, IL-8, IL-10, IL12, IFN- γ, TNF-α) areelevated along with levels of soluble CD14, lipopolysaccharide binding protein (LPB) and the complement component C5a (1). Potent chemoattractants such as CXCL8 and its receptors CXCR1 and CXCR2 are subjects of interest in relation in vaccine development. Variation in the innate immune response is a feature of different mastitis pathogens and strain types, with S.uberis demonstrating strain specific behaviour as either acute clinical cases or persistent sub-clinical IMI (27).

A role for cell-mediated immune responses via T cells in resistance to mastitis caused by *S. uberis* has been proposed in the quest for vaccines and lymphocytes may be important in co-ordinating the response to infection (13). Other approaches such as the induction of an antigen specific immunoglobulin A (IgA) response in the udder (9) and vaccination of dairy cows with recombinant *S.uberis* adhesion molecule (SUAM) which then interferes with binding to lactoferrin, have also been explored (30). The role for biofilms in the pathogenesis of *S. uberis* infections as well as *S.aureus* is also drawing the attention of researchers (45). Although, a commercial vaccine remains elusive.

COMMERCIALLY AVAILABLE MASTITIS VACCINE

The inactivated vaccine Startvac®, (HIPRA, S.A., Amer, Spain), is marketed in the UK for use against *E.coli*, coliforms, *S.aureus* and coagulase-negative Staphylococci and contains the inactivated *Escherichia coli* J5 strain and the *Staphylococcus aureus* SP 140 strain expressing Slime Associated Antigenic Complex (SAAC).

Indications:

“For herd immunisation of healthy cows and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence of sub-clinical mastitis and the incidence and the severity of the clinical signs of clinical mastitis caused by Staphylococcus aureus, coliforms and coagulase-negative staphylococci."

The full immunisation scheme as per SPC requires administration at 45 and 10 days pre-calving followed by a further administration at 52 days post-calving and induces immunity from approximately day 13 after the first injection until approximately day 78 after the third injection.

One dose (2 ml) contains:

Escherichia coli (J5) inactivated > 50 RED60 *
Staphylococcus aureus (CP8) strain SP 140 inactivated, expressing Slime Associated Antigenic Complex (SAAC) > 50 RED80 **

* RED60: Rabbit effective dose in 60 % of the animals (serology).
** RED80: Rabbit effective dose in 80 % of the animals (serology).
Liquid paraffin: 18.2 mg
Benzyl alcohol: 21 mg

Clinical trials carried out with STARTVAC on farms with 198 cows vaccinated and 188 unvaccinated control cows showed that vaccination reduced the incidence of clinical and subclinical mastitis and severity of clinical symptoms of mastitis caused by *S. aureus*, coliforms and coagulase negative staphylococci (CNS) (32). Robust assessment of the efficacy of mastitis vaccination requires large studies and work on a large randomised control trial is ongoing in this regard (37).

VACCINATION CHALLENGES

Compliance & concordance

Meadows described worryingly low compliance rates from farmers in BVDV vaccination in the UK (28). The label regime as detailed on the SPC for STARTVAC can be challenging to achieve; requiring administration at 45 and 10 days pre-calving followed by a further administration at 52 days post-calving. An alternative 'rolling' protocol has been proposed and evaluated (5), which follows the initial two dose primary course pre-calving with a rolling 3 monthly administration of vaccine.

Multifactorial farm management factors

Zadoks (44) and Breen (7) described the multifactorial risks for bovine mastitis. Narrowly focused vaccination programmes are likely to have significantly different impacts on commercial farms, depending on the prevailing epidemiology.

Minor pathogens

Coagulase negative staphylococci are gram positive cocci that inhabit both the inside of udder and live on teat skin. They are often described as opportunistic pathogens and include *S. chromogenes* and *S.epidermitis*. They are usually mild infections that may cause chronic elevations in SCC but often show spontaneous cure. Alongside *C.bovis* it remains unclear as to their significance as pathogens or potential protective role (21). Teat apex colonisation by CNS has been suggested to exert a protective effect against IMI caused by major pathogens. Competitive exclusion of other mastitis pathogens, production of inhibitory substances such as bacteriocins or stimulation of the innate immunity have been proposed as mechanisms of protection (11). Do we want to prevent or promote the presence of minor pathogens in the udder?

Emerging pathogens-prototheca

The high yielding udder is a tempting target for emerging opportunistic pathogens, such as mycoplasma or prototheca (33). It is likely that new challenges will continue to arise as we address old ones and therefore opportunities for further vaccine technologies will remain.

A BALANCED HOLISTIC APPROACH: DMCP

A bespoke approach to mastitis control is therefore required in every herd, including management of the environment, milking machine and routine and nutrition (39). The host pathogen interaction inevitably varies greatly, however, evidence based support tools and national campaigns for industry wide mastitis improvement such as the DairyCo Mastitis Control Plan (DMCP) have become established (18).

An opportunity exists to build on DMCP with exciting advances in diagnostics to a strain level, linked to targeted knowledge exchange through farm training programmes and bespoke herd health advisory input. Vaccination represents just one part of this joined up approach to mastitis control, but may have an increasingly important role to play in the future.

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ARE COAGULASE-NEGATIVE STAPHYLOCOCCI A PROBLEM?

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INTRODUCTION

Despite substantial research efforts, mastitis [inflammation of the mammary gland as a result of intramammary infection (IMI)] remains a costly, annoying, and imago-threatening disease for dairy farmers and the dairy industry.

A large number of microorganisms are causing IMI in dairy cows although staphylococci, streptococci, and coliforms cause the majority. As a result of the better control of clinical and subclinical mastitis caused by major pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, the bulk milk somatic cell count (SCC) has decreased over the last decades in many areas of the world. At the same time a shift towards a higher prevalence and incidence of IMI caused by the so-called environmental pathogens and coagulase-negative staphylococci (CNS) has taken place. Nowadays, CNS have even become the principal cause of subclinical mastitis on many dairy farms that have controlled contagious mastitis (e.g. 6), and are frequently found in the milk of (early lactating) heifers (4).

Conflicting results on aspects as impact on milk yield, epidemiology of and protection by CNS IMI, has led researchers to challenge the idea that CNS are a homogeneous bacterial group.

ECOLOGY AND EPIDEMIOLOGY

Since phenotypic methods to identify CNS isolates (from milk and other sources) often yield unreliable results (16), various molecular identification methods based on gene sequencing or fingerprinting techniques have now been developed (15). After giving special attention to library construction, fingerprinting methods generally outperform phenotypic ones and are the basis for studying differences between CNS species and strains. In addition to culture-based detection of isolates, culture-independent methods are of interest (2).

Overall, molecular methods are essential to track epidemiological patterns of CNS strains causing IMI and to gain deeper understanding of the population structure of the predominant CNS species found in milk. Such approaches have shown that typically five CNS species commonly cause IMI: *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus simulans* and *Staphylococcus xylosus* (14).

The current knowledge on the ecology and epidemiology of these species was recently summarised (15) and suggests *S. chromogenes* to be a cow-adapted species, with *S. chromogenes* IMI cases being generally of an opportunistic nature. Also *S. haemolyticus* appears to act primarily as an opportunistic pathogen but this species is capable of inhabiting highly diverse habitats. *Staphylococcus xylosus* seems to be a highly versatile species too; yet, very little is known on the epidemiology of cases of *S. xylosus* IMI. Although *S. epidermidis* is traditionally considered a human-adapted species, it is capable of residing in other habitats as well. Still, *S. epidermidis* IMI appear to emanate predominantly from human sources. *Staphylococcus simulans* might typically cause contagious IMI in a herd, although opportunistic *S. simulans* IMI cases also occur.

Interestingly, every herd seems to have its specific CNS microbiome, over different habitats such as teats, milk, the environment, and extramammary niches (1, 3, 10).

RELEVANCE

Inconsistent results on aspects such as the impact of CNS IMI on SCC and milk yield, the virulence potential but also protective aspects of CNS when causing IMI or when colonising the teats apices, have caused confusion regarding the true importance of CNS for udder health.

The CNS group has always been considered to be of minor importance for bovine udder health. Indeed, as a group, CNS increase the SCC moderately and have been reported as causes of mild cases of clinical mastitis only. There are indications that the typical effects, however, differ between CNS species and perhaps even strains. *Staphylococcus chromogenes*, *S. simulans*, and *S. xylosus* induce an increase in the quarter milk SCC that is higher than other CNS species (5, 13). The percentage contribution of CNS IMI to the bulk milk SCC can also be substantial on well-managed herds (12). It has also become clear that many CNS species can cause persistent IMI, contrary to what has long been believed (14).

Surprisingly, milk production is higher in CNS-infected heifers and cows compared to culture-negative animals (7, 9). Also, CNS infected heifers are less likely to develop clinical mastitis throughout first lactation, which is potentially related to a protective effect of pre-existing CNS IMI against new IMI with major pathogens. A recent met-analysis revealed that, overall, observational studies showed no such effect, whereas challenge studies showed strong and significant protective effects, specifically when major pathogens were introduced into the mammary gland via methods bypassing the teat end (11).

Besides CNS IMI, teat apex colonization by CNS has been suggested to exert a protective effect against IMI caused by major pathogens (8). Potential mechanisms could be competitive exclusion of other mastitis pathogens, production of inhibitory substances such as bacteriocins, or stimulation of the innate immunity, or most likely a combination.

CONCLUSIONS

Lots of exciting CNS research using molecular differentiation of species is currently being undertaken revealing what was hypothesized before: difference between CNS species exist and are relevant. Some species are more adapted to the mammary gland than others, affect udder health differently, are more likely to carry virulence and/or resistance genes … with many more differences between species and within species to be detected.

Coagulase-negative staphylococci are not likely to be a problem on whatever dairy herd, yet might need some attention on well-managed dairy herds were the major mastitis pathogens are well-controlled, especially when the more virulent CNS species are involved.

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REDUCED SOMATIC CELL COUNTS IN RESPONSE TO A STANDARDIZED HIGH ACTIVITY PROPRIETARY GARLIC POWDER ADMINISTERED TO LACTATING COWS – A PILOT TRIAL

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INTRODUCTION

Elevated somatic cell counts (SCC) during lactation could only be treated at the end of lactation with long acting intramammary preparations, usually with a host of antibiotics. Any attempt to treat the cow during lactation would mean discarding the milk. A herd with a persistently high SCC results in poor quality milk and lower returns for the farmer. In view of these issues, natural alternatives are constantly sought to better the health of cows and increase returns to the farmer.

METHOD

A Standardized High Activity Proprietary Garlic Powder (IQP-AS-106-1) was administered in different concentrations to lactating cows in a research farm in New Zealand to assess its efficacy in lowering SCC in milk. Eighty cows with composite SCC over 300,000 cells/mL were selected and a random, stratified method was used to allocate them into 4 groups of 20 cows each. All groups at start of study had equal means. Quarters on these cows that were over 300,000 cells/mL were observed throughout the study. Group 1 was the control and was given water. Each cow in Groups 2, 3 and 4 received 150 mL, 300 mL and 450 mL respectively of IQP-AS-106-1 dissolved in water. All cows were dosed for 5 days. SCC analyses were performed from the milk samples of all cows until 28 days after the last day of dosing. The efficacy of the dosing was evaluated by calculating the geometric means of the SCC in the observed quarters before and after dosing.

RESULTS AND DISCUSSION

Group 3 showed the best response with a 61% reduction of SCC after the last day of dosing versus 46% and 54% reduction in Groups 2 and 4 respectively. Meanwhile the control group had only 41% reduction of SCC. After just 3 days of dosing, Groups 3 and 4 had their SCC reduced to below 300,000 cells/mL. No adverse events were reported during the study. This study showed that at 300 mL, IQP-AS-106-1 (a component in Vetrinol) was able to lower high SCC in cows during lactation to less than half of their starting SCC. Thus, further investigations in a larger study seem to be warranted, in order to show herd-wide effects.

INVESTIGATION OF INHIBITORY SUBSTANCES FOUND IN MILK – PRELIMINARY REPORT FORM

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SUMMARY

BCVA has produced a revised and updated version of the Inhibitory Substance in Milk Investigation Form.

This form is designed to be used by anybody involved with investigating a bulk tank inhibitory substance failure – vets, milk purchasers, milk processors, pharmaceutical companies and other industry bodies and consultants.

The form provides guidance for collecting all relevant farm information regarding the bulk tank inhibitory substance failure as well as about the milking animals on the farm at the time of the failure.

The form is available in two formats:

- PDF for printing
- Locked word document for completion electronically

To download the form please use the BCVA website:

www.bcva.org.uk

or email the BCVA Office:

office@cattlevet.co.uk.

The form is reproduced in the Appendix of these Proceedings.

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SEPARATED MANURE SOLIDS AS BEDDING FOR DAIRY COWS – A UK FARMER SURVEY

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INTRODUCTION

Increased costs and reduced availability of traditional bedding sources have prompted many dairy farmers to search for alternatives. Slurry separation technology is nothing new, but the way it can now be used is. Separators now have the ability to extract more liquid, to produce material with dry matter levels of 34-38%. As a result, interest has grown in the separation of manure for the production of cattle bedding, but in the absence of information on performance in UK conditions.

METHODS

Users of separated or "recycled" manure solids bedding (RMS) were identified through contact with advisors, veterinary surgeons and machinery distributors and word of mouth. In autumn 2013, a telephone questionnaire was conducted to obtain information on the practices and experiences of early adopters of the approach on dairy farms in the UK. The 19 farmers who responded were contacted again after 3-6 months to determine whether they were still using the bedding.

Samples of unused and used bedding were submitted by 18 farmers for bacteriological analysis. Putative counts of coliforms (VRB media), *Streptococci* (Edwards agar) and *Staphylococci* (Baird Parker media) were made.

RESULTS

The 19 respondents had been using the bedding for between 6 weeks and 3 years (mean 9 months). Herd size ranged from 180 to 1200 cows (mean 413). Seven farms used RMS on mattresses, four on deep beds, and ten had tried some of each. Four used the material in loose yards or pens. One composted the material before use.

Bacterial counts were typically high in fresh material (Coliforms 10^4-10^6cfu/g, *Streptococcus* spp. 10^7-10^8cfu/g, *Staphylococcus* spp. 10^3-10^6cfu/g). Bacterial numbers tended to increase with use, though most markedly for the coliforms. Counts in used material did not differ between shallow and deep beds.

The benefits most commonly mentioned in response to an open question were: cost savings compared with alternatives (previously 12 sawdust, 4 sand, 3 paper), ease of slurry storage and handling, cow comfort including increased lying times, cow cleanliness, availability, making it easy to use bedding liberally, and reduced dust in buildings. Udder cleanliness, improved utilisation of slurry, and reduced hock lesions were mentioned by three or four farmers.

Challenges mentioned were: achieving consistent dry matter product, more management input required, cell count/mastitis problems when bedding is damp and cell count/mastitis problems when waste milk was included in slurry. Farmers' suggestions to address these challenges are shown in Table 1. Difficulties in achieving or maintaining dry enough bedding, leading to udder health problems, caused three users to discontinue use after 5, 8 and 13 months respectively.

CONCLUSIONS

Early adopters of separated manure solids as bedding commonly perceived benefits in terms of cost, convenience, cow comfort and cleanliness. Difficulties in achieving or maintaining dry enough bedding led to three users discontinuing the practice in the face of udder health problems.

Evidence gaps still exist on the risks to cow health and further research is required, particularly on treatments for bedding that might reduce these risks.

Table 1. Farmer reports of challenges of using separated manure solids as bedding and farmers' suggestions for solutions

Challenges (collated from open questions)	Farmer solution	No of farms
Achieving consistent DM product	Stirring, ensuring consistent product entering separator	5
Achieving consistent DM product	Machine maintenance – checking and replacing sieves	4
More management input required	Include time for monitoring, be observant, train staff	4
Cell count/mastitis problems when bedding is damp	Do not separate outdoors, do not use damp bedding, do not use in poorly ventilated buildings	3
Cell count/mastitis problems when waste milk is included in slurry	Divert waste milk	2

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OBSERVATION OF MASTITIS PARAMETERS IN FARMS USING STARTVAC®. AN UPDATE AFTER 2 YEARS.

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INTRODUCTION AND AIMS:

Monitoring the dynamic nature and magnitude of the mastitis challenge, prevalence and incidence within the modern dairy herd is becoming common place around the developed dairy industries of the world. The iterative process of regular interpretation of prevalence data (Individual Cow Somatic Cell Counts [SCC]), incidence data (Clinical mastitis records) and how these data change over time both within lactation and between lactations (over the dry period) can give much information about the transmission patterns and likely origin and duration of intramammary infections within a dairy herd. The transmission patterns can indicate whether new intramammary infections tend to be persistent and thus have the opportunity to spread in a contagious manner or transient and more likely to originate from the cows environment. The stage of lactation that new intramammary infections occur within a herd, either as increases in cow SCC above a threshold or the occurrence of a clinical episode can help indicate their presumptive origin from the dry period or lactation.

Overall this approach is increasingly being used to help indicate the likely source and magnitude of the mastitis infection drive and spread within a dairy herd such that management advice can be given to control intramammary infection rate. The same approach can be used to measure the effects of interventions such as changes in management protocols for example improved transition cow management, improved milking routine or vaccination.

The initial aim of this project was to apply these diagnostic and monitoring techniques to three herds chosen for a high prevalence of Staphylococcus aureus over the 12 months prior to and during the 12 months of a rolling 3 month vaccination program with a polyvalent mastitis vaccine.

Startvac'(Hipra) is an inactivated oil adjuvented vaccine containing Escherichia coli J5 and Staphylococcus aureus (CP8) strain SP 140 expressing Slime Associated Antigenic Complex (SAAC). After this initial observational period two of the herds decide to continue with the vaccination program.

MATERIALS AND METHODS:

Data is compared at 6 month intervals from 12 months prior to and 24 months after initiating a whole herd Startvac® vaccination policy. All cows received 2 vaccine doses 3 weeks apart followed by a rolling policy of quarterly boosters. In calf heifers were batched to receive 2 doses 3 weeks apart with the second dose no less than 10 days prior to expected parturition. Heifers then joined the rolling 3 month booster program.

RESULTS AND DISCUSSION

First 12 months (3 farms vaccinating)

The parameters observed were:

- *Staphylococcus aureus* prevalence - using individual cow bacteriological culture of all cows in milk before and after the project.
  - Reduction in *Staphylococcus aureus* prevalence in all 3 herds by 71%
  - The potential impact of culling of known positive *Staphylococcus aureus* animals was studied
- Clinical mastitis incidence.
  - Reduction in clinical mastitis incidence in all 3 herds by 54%
- Routine monthly individual cow SCC data – using a 200,000 cells per ml threshold to indicate infection status.
  - Reduction in proportion of infected cows in the herd (% cows > 200,000 cells per ml) by 4%
  - Reduction in First infections in the 3 herds (high 1st cell count after parturition) by 71%. This effect was seen in both cows and heifers
- Reduction in New infections in two herds and increase in one herd (1st high SCC in current lactation but not 1st SCC after parturition)
- An estimation of cost benefit for the 12 month period of vaccination for the 3 herds was six times the cost of the vaccine.

First 24 months (2 farms vaccinating)

- Clinical mastitis incidence: both herds had an increase in clinical mastitis in the 12 to 18 months period after initiating vaccination. They had a likely lactation environmental outbreak of clinical mastitis. But they have subsequently returned to the improved mastitis rates seen subsequent to vaccination.
- The reduction seen in the first 12 months after vaccination for the proportion of infected cows and First infections was maintained in the second 12 months.

CONCLUSION

In this longitudinal study during two years of use of STARTVAC an improvement in the clinical mastitis, Staphylococcus aureus prevalence and individual cow somatic cell count was observed. It is important to remark that better results are obtained after 6 months using the rolling protocol - in common with other studies as the improvements take time to accumulate. Vaccination is a valuable tool to control *Staphylococcus aureus* infections but it is important to remember that as with any control tool, vaccination is not a panacea and consistent good mastitis management is essential.

NOTES

NOTES

APPENDIX

INVESTIGATION OF INHIBITORY SUBSTANCES FOUND IN MILK – PRELIMINARY REPORT FORM

BRITISH CATTLE VETERINARY ASSOCIATION

Investigation of inhibitory substances found in milk – preliminary report form

Name and Address of Veterinary Surgeon		**Name and Address of Farmer**	
Milk purchaser		**Membership number**	
Date of Failure		**Quality testing lab**	
Date of investigation		**Investigation method**	
Milking Plant			
Type / size of parlour			
Computerised / Auto Id		**YES / NO**	
Was separate dump line used?		**YES: dump bucket / line / dump cluster / NO**	
Milking routine			
Who did the milking?			
Were relief milkers milking on day of failure?		**YES / NO**	
How are cows identified? (Freeze brand / ear tag / Auto Id etc)			
How are cows under withdrawal identified?			
Were cows under withdrawal clearly identified at the time of the failure?		**YES / NO**	
Is there a marker board in the parlour?		**YES / NO**	
Are cows under withdrawal milked last?		**YES / NO**	
Are quarter milkers used?		**YES / NO**	
Frequency of milk collection		**DAILY/ EVERY OTHER DAY/ OTHER**	
Bulk tank volume on day of failure		**L**	
Animal treatments			
Are medicine records up to date?		**YES / NO**	
Are dry cows separated from the milkers?		**YES / NO**	

Did any new calved cows have Milk Fever?	YES / NO
Were any cows purchased in the 10 days prior to the failure?	YES / NO
How many cows were milked on day of failure?	
Were any cows treated during the milking routine? If yes, were hands washed after handling antibiotics?	YES / NO YES / NO
Were any cows treated 'off label' at the time of the failure? If 'yes' – what milk withdrawal period was advised?	YES / NO / DON'T KNOW Advisory withdrawal period:
Is a teat sealant used?	YES / NO
Are medicines stored securely?	YES / NO
Could anybody have interfered with the bulk tank?	YES / NO
Has the vet been attending any animal recently?	YES / NO
Is there a test kit available?	YES / NO

Individual cow tests carried out for antibiotics

Cow Id.	Date	Test performed	Result	Container type used for collection of milk	Batch no and expiry date of test

Antibiotics routinely used on farm to milking animals

Product	Reason for use

Animal treatments to milking cows in the 10 days prior to the failure					
Cow Id.	**Treatment (product name)**	**Dose**	**Date**	**Date milk added to bulk tank**	**Office Use Only**
	SYSTEMIC ANTIBIOTICS				
	INTRAMAMMARY ANTIBIOTICS				
	ANTHELMINTICS				
	OTHER				

New calved cows (including early calved/aborted) in the 10 days prior to the failure						
Cow Id.	**Dry cow treatment date**	**Treatment (product name)**	**Dose**	**Calving date**	**Date milk added to bulk tank**	**Office Use Only**

Sampling for inhibitory substance test

1. Ensure that the sample is representative of milk from *all quarters* (ie composite of equal volumes)
2. Always use an approved sample container that is clean and dry and does not contain any preservatives or inhibitors
3. If collecting from a recorder jar, ensure it has not been contaminated from a previous animal
4. Avoid contaminating the sample with inhibitory substances on collection(eg disinfectants, teat dips)
5. Ensure the sample is correctly identified, labelled and securely sealed
6. Ensure the sample is kept cool, preferably frozen, between collection and testing
7.

Refer to manufacturer's instructions for individual test protocol if appropriate