# BRITISH MASTITIS CONFERENCE 2025

**Wednesday 18th June 2025**
**5 Star Window Suite, Sixways Stadium, Warriors Way, Worcester, Worcestershire, WR3 8ZE**

## Organised by

The Dairy Group | British Cattle Veterinary Association | The University of Nottingham UNITED KINGDOM · CHINA · MALAYSIA | QMMS

## Topics:

- Role of contagious mastitis
- Association between genomics and mastitis
- Knowledge transfer & Research updates
- Milk quality in Rwanda
- Housing design for a changing environment
- A mastitis control case study

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# GENERAL INFORMATION

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# CHAIRMAN'S INTRODUCTION

Welcome to the 2025 and 37th British Mastitis Conference at Sixways Stadium, Worcester.

The organising committee has again been guided by delegate feedback and we believe that we have brought together a group of speakers from the UK, Ireland, mainland Europe and the USA which will provide interesting, thought provoking and stimulating presentations. We have tried to strike a balance between up-to-date research results and practical presentations with clear take home messages.

The first paper looks at the role of contagiousness in mastitis control. This will be followed by a paper on the association between genomics and mastitis. We will then have a short break for tea and coffee with time for delegates to look at the posters and ask questions of the presenters.

The Knowledge Transfer / Research Update section is an important part of BMC. We have selected four posters from those submitted for oral presentation. The four papers are followed by an opportunity for delegates to debate with each of the presenters.

After lunch there will be a presentation on milk quality in Rwanda, which will be followed by a paper on housing design for a changing environment. The final paper at BMC 2025 will be the ever-popular Mastitis Control Plan case study.

This year we have seen a number of "new faces" presenting. The nine posters cover a wide range of topics with the common theme of improving the mastitis levels in dairy cows together with overall milk quality. Please take time to review the posters and speak with the authors. Thanks to all poster presenters who have put a great deal of effort into providing the abstracts and preparing and presenting their posters, so please do read their work and vote.

We endeavour to find you the best speakers with the most relevant (and latest) information. This is only achievable thanks to the generous support of all our sponsors. This year our sponsors are: ATL Agricultural Technology Limited (Gold), Vetoquinol (Gold), Ambic (Silver), Boehringer Ingelheim (Silver), Milkrite I InterPuls (Silver), Phibro Animal Health (Silver), CID Lines (Silver), ADF Milking Limited (Bronze), Zoetis (Bronze) and Oxi-Tech Solutions Ltd (Best Poster Competition).

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

Finally, thank you for attending and supporting the conference. I trust you will have an enjoyable and worthwhile day and we hope to see you at our 38th BMC in 2026.

Ian Ohnstad, British Mastitis Conference Chairperson, *The* Dairy Group

# TIMETABLE OF EVENTS

| | | |
|---|---|---|
| **08.45** | **ARRIVE / REGISTRATION / COFFEE & TEA AND POSTER DISPLAY** | |
| | | |
| **09.45** | **CHAIRMAN'S INTRODUCTION** | **Ian Ohnstad**<br>The Dairy Group, UK |
| | **Session One** | **Brian Pocknee**<br>DHC, Spain |
| **09.55** | The role of contagiousness in mastitis control | **Peers Davies & Andrew Bradley**<br>Universities of Liverpool & Nottingham, UK |
| **10.30** | Association between genomics and mastitis | **Marco Winters**<br>AHDB, UK |
| | | |
| | **Session Two**<br>**Research Updates / Knowledge Transfer (also presented as posters)** | **Elizabeth Berry**<br>BCVA; UK |
| **11.40** | Chlorine dioxide farm water treatment reduces mastitis rate | **Phil Elkins**<br>Farm Water, UK |
| **12.00** | Utilisation of multiple sample protocols to facilitate Genocells® somatic cell count measurement in large herd scenarios | **Richard Miller**<br>NMR, UK |
| **12.20** | Factors associated with bulk tank SCC on Irish dairy farms | **Alice Uí Chearbhaill**<br>Teagasc, Ireland |
| **12.40** | Precision vacuum control in conventional and automatic milking installations | **Doug Reinemann**<br>University of Wisconsin, USA |
| | | |
| **13.00** | **LUNCH & POSTERS** | |
| | | |
| **14.10** | **WELCOME BACK & VOTING ON POSTERS** | |
| | | |
| | **Session Three** | **Brian Pocknee**<br>DHC, Spain |
| **14.15** | Milk quality in Rwanda | **Ian Ohnstad**<br>*The* Dairy Group, UK |
| **14.50** | Housing design for a changing environment | **Zoe Barker**<br>University of Reading, UK |
| **15.25** | Mastitis control plan case study | **Emmie Bland**<br>Yan Farm Health, UK |
| | | |
| **16.00** | **POSTER AWARD** | |
| | | |
| **16.05** | **CLOSE** | |

# Titles of Papers and Presenters

# Titles of Posters and Authors

# FURTHER INFORMATION

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

**Organising Committee**

**Chairman: Ian Ohnstad**
**Conference Secretariat: Karen Hobbs & Anne Sealey**
**Editor: Brian Pocknee**

**Scientific Committee**

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A global organization for mastitis control and milk quality

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

## What does NMC do?

- Provides a forum for the global exchange of information on mastitis and milk quality
- Publishes educational materials
- Establishes guidelines for mastitis control and milking management practices
- Monitors technological and regulatory developments relating to udder health, milk quality and milk safety
- Conducts meetings and workshops, providing educational opportunities for all segments of the dairy industry
- Offers a Scholars program for graduate students

*A commitment to reducing mastitis and enhancing milk quality*

## Who are the members of NMC?

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

## What can NMC do for you?

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

## Why join NMC?

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

## NMC membership benefits

- NMC annual meeting and regional meeting proceedings, containing all of the papers and posters presented at the meetings
- NMC electronic newsletter, 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, NMC membership directory, and NMC Job Board
- 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.*

## Working together

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

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# PAPERS

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*Proceedings of the British Mastitis Conference (2025) Sixways, Worcester, p 1 -13*
*The Dairy Group, The University of Nottingham, BCVA & QMMS*

# THE ROLE OF CONTAGIOUSNESS IN MASTITIS CONTROL

**Peers L Davies[1] and Andrew J Bradley[2,3]**
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## SUMMARY

Humans love to categorise the world around them; it is a very useful cognitive shortcut to simplify the complexity and nuance of the natural world into a framework which can be understood and acted upon. The way in which we have approached bovine mastitis control over the past seventy years is a good example of how categorisation in the decision-making process can be very successful, at least initially, and also illustrates how pathogens can exploit and adapt to our categorical control measures to thrive in ecosystems we have created. In this paper we briefly discuss the historical perspective of mastitis control and some of the more recent evidence challenging the appropriateness of the traditional categorisations, and how we might address contagious transmission in the future, with a particular focus on *Streptococcus uberis*. Finally, we will consider approaches to categorising mastitis on farm, based on disease patterns and sub-species strain typing rather the pathogen species alone.

## Historical perspective

In the 1970's the principal mastitis pathogens in the UK dairy herd were *Streptococcus agalactiae, Streptococcus dysgalactiae* and *Staphylococcus aureus,* accounting for 58.1% of diagnoses [3]. All of these pathogens were believed to reside principally or exclusively in the mammary gland or on the skin and mucous membranes of the cow rather than in the environment. In contrast *Streptococcus uberis* and *Escherichia coli* combined only accounted for 9% of diagnoses. These two pathogens had been shown to be present in the gastrointestinal tract of ruminants and in the farm environment (housing and pasture) [19,21,32]. In the 1960's and 70's identification of pathogens to the species level was the practical limit for diagnostic use, although sub-species strain typing was being developed for research purposes.

## 'Contagious' vs 'Environmental' mastitis classification

The 'Contagious' vs 'Environmental' classification system for mastitis transmission within a herd has been the dominant paradigm in mastitis research

and clinical veterinary practice since at least the 1960's although no clear attribution could be found for the first definition. The system hinges upon discriminating pathogens based upon their ability to persist and multiply in, or on, the mammary gland in strong preference to other sites. *S. agalactiae* being the most extreme example of this 'cow-adapted' behaviour. The key biological attribute is the ability to persist for a prolonged period of time in the udder, shedding bacteria into the milk and persisting in milking equipment. This increases the number of contagious transmission opportunities to infection of the next host. In contrast the opportunistic 'environmental pathogen' has not evolved to explicitly exploit the dairy cow in this way. It is by contrast a flexible creature, able to exploit a wide range of ecosystems including the mammary gland. By extension many of the interactions we see in other host-pathogen relationships we also see in bovine mastitis. Specifically, the host-adapted pathogens tending towards stimulating a less aggressive immune response than the opportunistic invader as demonstrated by de Haas *et al* [11]. While de Hass *et al* [11] did show significant differences in somatic cell count (SCC) recovery patterns between bacterial species it was not a perfect separation, indicating that some infections due to presumed 'environmental' species could stimulate the long duration SCC response pattern associated with cow-adapted, contagious pathogen species (Fig 1).

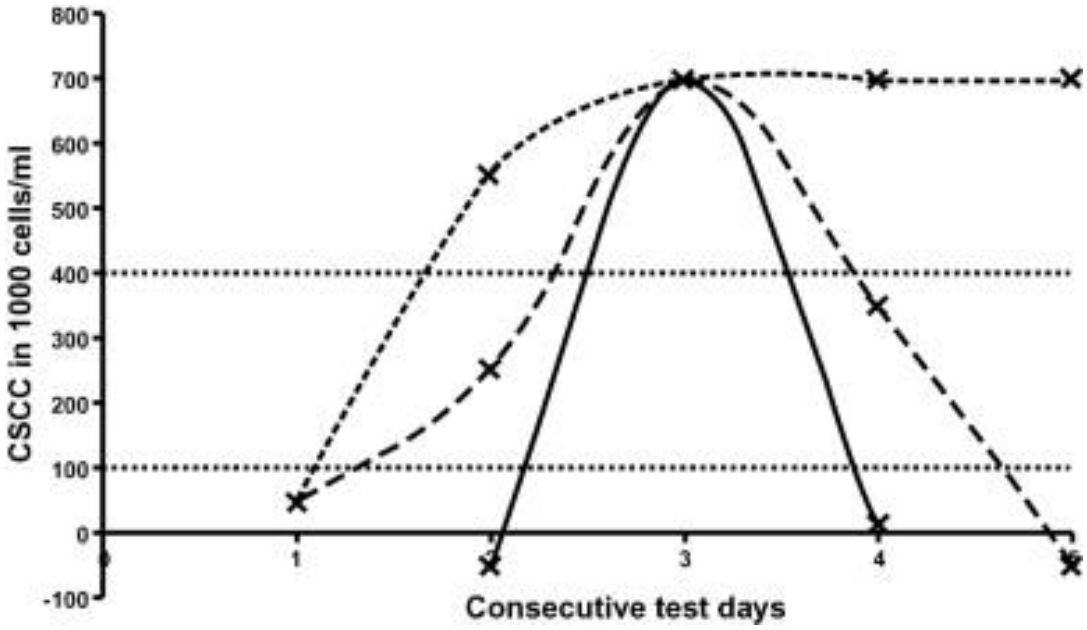


**Figure 1. The solid line shows an example of a short, quick increase in SCC, relative to SCC for lactations without elevated SCC (CSCC), i.e., the "quick recovery pattern." The broken line shows an example of a slow increase in SCC, but with recovery within 5 consecutive test days, i.e., the "slow recovery pattern." The dotted line shows an example of a long increased SCC, without recovery within 5 consecutive test days, i.e., the "no recovery pattern." This figure reproduced from de Haas *et al* (2004) [11].**

There is a clear attraction for both farmers and vets to classify the route source of mastitis infections in each herd according to the species of the principal pathogen cultured from milk samples. This species level approach is quick, simple and inexpensive as it relies upon culture and species level identification from a small number of milk samples. It has also been undoubtedly a useful broad classification system for reducing mastitis incidence in herds with previously poor mastitis control [28]. However, it is inherently a radical simplification of the biological diversity found in mastitis causing pathogens. When applied too rigidly it can hinder a more nuanced understanding of mastitis epidemiology and frustrate attempts to control the disease. The best evidence to support the validity of the 'Contagious' *vs* 'Environmental' classification system is by correlation with the implementation of control measures designed to reduce either contagious, cow-cow transmission, such as the Five Point Plan or measures designed to prevent bacterial invasion from the environment such as pre-milking teat disinfection (preMTD). In the 1960's the very high incidence rate of infection in the UK national herd of 153 clinical mastitis cases per 100 cows per year [29] presented a relatively easy baseline from which rapid improvements could be made. In the 1960's 58% of clinical cases were attributed to pathogens classified as 'Contagious'; *S. aureus, S. agalactiae* and *S. dysgalactiae* whilst only 9% were attributable to 'Environmental' pathogens *S. uberis* and *E. coli* [29]. The Five Point Plan was introduced to address this problem and focused on measures that were highly likely to reduce the number of chronic mastitis cases and reduce the risk of transmission via the milking equipment. This resulted in a dramatic reduction in the overall mastitis rate to 40 cases per 100 cows per year in 1982 [28] and a reduction in the proportion of cases attributed to 'Contagious' mastitis from 58% to 30%. However, the relative proportion attributed to 'Environmental' mastitis increased from 9% in 1967 to 48% in 1982 [28].

The emerging dominance of *E. coli* and *S. uberis* in mastitis diagnoses during the 1970's – 80's led to an emphasis on the environmental hygiene of bedding during housing periods and at pasture [7,8,24]. However, in the UK and USA in particular, much attention was focused on pre-milking teat disinfection (preMTD) as a control method for 'Environmental' mastitis. This was based upon the broad assumption that the pathogen resided and multiplied off the host or at least away from the udder (eg *E. coli* in the GI tract). Therefore, control was by minimising contact with and contamination of the teats and udder between milkings. Unlike the Five Point Plan for 'Contagious' mastitis there has not been such clear evidence of successful control measures for 'Environmental' mastitis. Many studies have investigated the efficacy of preMTD with a range of different active ingredients. Field trials have identified a significant reduction in mastitis associated with the practice [18] and a smaller UK study demonstrated a non-significant reduction in clinical mastitis with preMTD [12]. However, several large studies which have failed to show any improvement in mastitis control with this practice [12,22]. In a more recent randomised controlled trial of preMTD under

grazing conditions in Australia where *S. uberis* was the dominant pathogen (accounting for 47% of all clinical mastitis (range 20%-97%)) there was a correlation between udder cleanliness and the efficacy of preMTD to reduce 'Environmental' mastitis [17]. In those herds managing cows in clean, dry conditions there was no additional benefit to preMTD in the clinical mastitis incidence. These studies indicated a stubborn disease challenge that was unresponsive to additional 'Environmental' control measures once gross contamination and cleanliness had been addressed. This relationship between cleanliness and preMTD efficacy was not correlated with the mastitis incidence at herd level [17]. The results indicate that high incidence rates of supposedly 'Environmental' *S. uberis* clinical mastitis (equivalent of >40 cases per 100 cows per year) were not controlled by either clean, dry conditions or pre-milking teat disinfection (preMTD) for 'Environmental' mastitis control. There was also an assumption that 'Contagious' control measures were entirely ineffective against all 'Environmental' pathogens. However, this was challenged when Wilesmith *et al* [28] found an average mastitis incidence over 3 years due to *S. uberis* of 20.8 cases per 100 cows per year. However, by 1982 the average incidence of *S. uberis* mastitis was 7 cases per 100 cows per year suggesting a substantial reduction in *S. uberis* clinical cases, alongside large reductions in the classic 'Contagious' pathogens; *S. aureus, S. agalactiae* and *S. dysgalactiae,* whilst there was no reduction in the incidence of *E. coli* mastitis over this period. This suggests that the Five Point Plan was partly effective in controlling *S. uberis* mastitis, indicating responsiveness to classic 'Contagious' transmission control measures. In contrast there was no such reduction in the incidence of mastitis caused by *E. coli* over the same period, suggesting that *S. uberis* and *E. coli* are not equivalent 'Environmental' mastitis pathogens, undermining the assumption that categorising herds mastitis transmission epidemiology based upon the presence of species alone was sufficient.

**Significance of *Streptococcus uberis***

*Streptococcus uberis* has been repeatedly identified as the most commonly isolated pathogen from clinical and sub-clinical samples in several countries including the United Kingdom, Australia, New Zealand and Belgium [31, 10, 23, 25]. In the UK *S. uberis* mastitis has become more common both in absolute and relative terms in the two decades following the introduction of the Five Point Plan, accounting for 23.5% of all clinical mastitis cases and approximately one third of sub-clinical, high somatic cell count (HSCC) diagnoses [4]. In the UK context *S. uberis* may have come to occupy the 'mammary ecological niche' made vacant by the relatively effective control of the other major pathogens through the implementation of the Five Point Plan. It is also possible that changing management practices within the dairy industry over this time period produced conditions more conducive to *S. uberis,* such as increasing herd size, changing housing and bedding management and potentially changes in genetic selection. Whatever the underlying causes for this emergence of *S. uberis* as the most

prevalent mastitis pathogen. It was identified as the single most significant barrier to achieving any further substantial reduction in the incidence of clinical mastitis in commercial UK dairy herds and therefore justifies disproportionate investigative interest [3,15,27].

Specific contagious control interventions have been identified to be efficacious in the control of *S. uberis* in particular; Wesen and Schultz, (1970) described a substantial and comparable reduction of 53% in the new mastitis infection incidence during lactation due to *S. uberis* and *S. aureus* in the trial quarters (RF & RH) of 125 cows undergoing post-milking teat disinfection with an iodine based preparation compared to a control quarters (LF & LH) [26]. In 2001 Zadoks *et al* [23] described a significant reduction in *S. uberis* intramammary infection incidence during periods when post-milking teat disinfection was practised in a herd experiencing high incidence of *S. uberis* mastitis and also referred to unpublished data indicating that *S. uberis* was recoverable from teat liners for up to two cow cycles after an infected (shedding) cow was milked with that teat cluster [30]. Given that the minimum infectious dose of *S. uberis* has been established experimentally to be <1000 cfu/ml [15] and that infected cows can shed > $10^6$ – $10^7$ cfu/ml it is plausible that the observed reduction in *S. uberis* incidence was causally linked to the introduction of post-milking teat disinfection. However, the efficacy of post-milking teat disinfection to prevent very small numbers of bacteria being inoculated into unaffected mammary glands may not be sufficient to entirely control contagious transmission.

Treatment of clinical cases of mastitis is fundamental to the control of contagious transmission as the antibiotic treatment curtails the infectious period of infective cows. In a true 'Environmental' mastitis pattern where each clinical case is acquired only from the environment each cow is a separate unit in a shared infective environment. This means that antibiotic treatment of any individual should not affect the infectious risk or mastitis incidence in other cows in the herd. A case report in 1996 was one of the first incidences where a contagious type mastitis pattern was linked to *S. uberis* [6] and a change in the antibiotic treatment protocol. A dramatic increase in the incidence of *S. uberis* clinical mastitis from 20% of clinical cases to 73% of clinical cases and a simultaneous exponential rise in the bulk milk somatic cell count occurred in the herd which had suspended antibiotic treatment of all clinical mastitis cases [6]. The *S. uberis* mastitis outbreak resolved following the reintroduction of clinical mastitis antibiosis. This case report has been cited as an example of a clinical mastitis pattern that did not fit the expected pattern of an opportunistic environmental pathogen [30].

In order to investigate the epidemiology of *S. uberis* mastitis it is essential to understand the population structure of the pathogen within a herd. This requires sufficiently discriminatory sub-species typing of bacterial isolates. In 2017 Davies *et al* published multilocus sequence typing (MLST) analysis of *S. uberis*

strain heterogeneity from 52 herds [9]. The study revealed a small subset of nine *S. uberis* strains of the several hundred known strains, that were disproportionately over-represented in multiple cows in a manner that might be explained by contagious transmission. The study showed this potentially mixed pattern of transmission across most of the herds, ranging from environmental dominant to contagious dominant patterns (Fig 2).

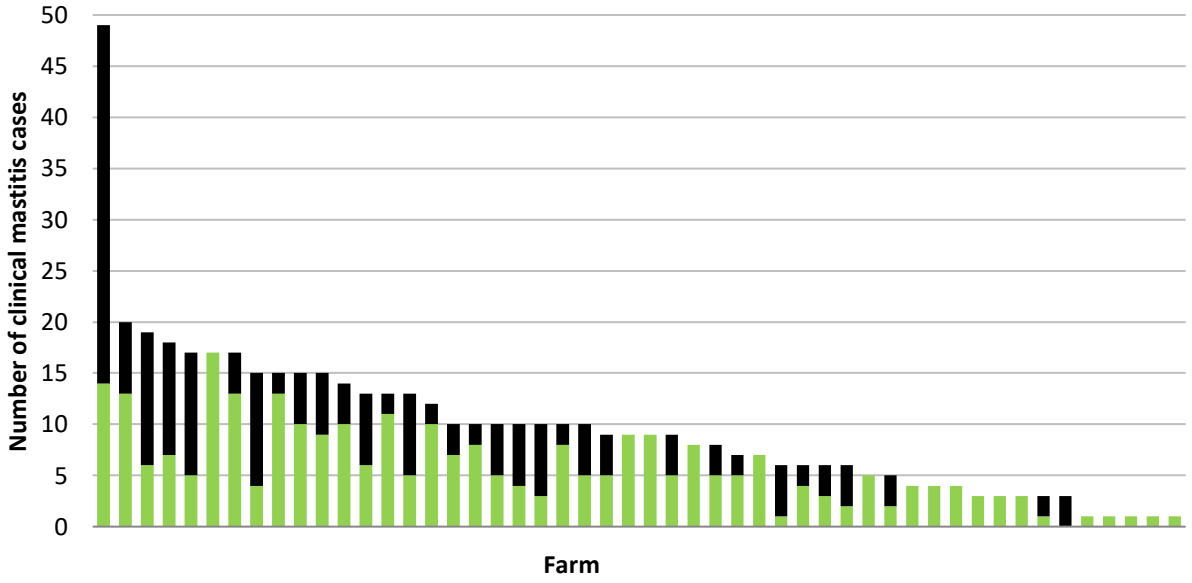


**Figure 2. Mastitis clinical cases by classification group for each farm where *S.uberis* mastitis cases were identified Green (environmental origin), Black (potentially contagious origin) reproduced from Davies *et al*, (2016) [9]**

In addition to the significant overrepresentation of these potentially contagious stains in case occurrence, the timing of the cases differed significantly also. These overrepresented strains occurred more evenly throughout lactation whereas the solitary strains which we expect reflect the diversity of strains in the environment were disproportionately likely to cause mastitis in the first 30 days and therefore associated with infection in the dry period, rather than during the lactation risk period for contagious mastitis. These two findings support the hypothesis that some strains of *S. uberis* are capable of contagious transmission and this ability is very likely to be additional to their ability to act as environmental pathogens. The evolutionary selection pressure which the pathogens experience when the immune system responds to their presence and when we intervene with treatments is clearly substantial and it is therefore not surprising that pathogens evolve to cope with these challenges. It would appear that *S. uberis* may be able to behave, in some instances, in a similar way to its classically contagious streptococcal relatives. This raises very interesting biological questions: is this convergent evolution where traits are independent or

have fitness genes been transmitted between closely related species during co-infections, for example?

**Discriminatory power**

Even with the strain level information provided by a traditional MLST scheme of seven highly conserved 'housekeeping' genes we still see a significant level of uncertainty at the individual farm or cow level. In order to fully understand if a mastitis case is due to contagious or environmental transmission we need even greater discriminatory power. To achieve this the authors subjected a subset of the potentially contagious strains from the previous study [9] to a whole genome sequencing in order to generate a more detailed 'core genome' MLST scheme (cgMLST) of over 1858 loci (unpublished data). This vastly increased discriminatory power allows the degree of relatedness between isolates from separate cows and cases to be quantified by the number of single nucleotide mutations as shown in Fig 3.

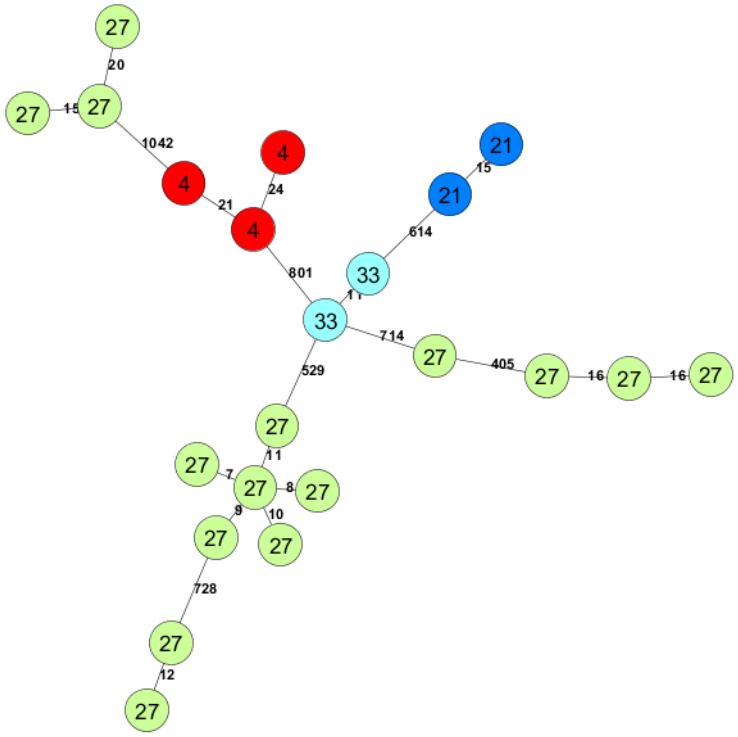


**Figure 3 . Minimum spanning tree of strain type 6 (ST6) isolates from Farms 4, 21, 27 & 33. Line distances are scaled log base 10 representing substitutions per nucleotide. Nodes are colour grouped according to farm of origin and label nomenclature is Farm-ST. Significant diversity is evident with the Farm 27 population with three clearly separated clades and a cluster of isolates from six separate mastitis cases indicating very close relationship (red circle).**

The high recombination rate of *S. uberis* provides a mechanism for individual bacteria to exchange very significant portions of their genome within an ecosystem. This recombination and exchange of genetic material could be in the form of either gDNA, plasmids, prophage or CRIPR's but by which ever mechanism, the effect on the epidemiology of *S. uberis* mastitis could be profound.

**Herd management**

Dairy herd management provides a number of opportunities for *S. uberis* to adapt and evolve into a new niche as illustrated in Fig 4. The dairy cow "provides" *S. uberis* with multiple different environments, each with their own characteristics. Of these the mammary gland and the gastrointestinal tract are the two most potentially significant. The gastrointestinal tract provides an ideal environment for the maintenance of a large and diverse population of *Streptococcus spp.* which is an ideal environment for widespread exchange of genetic material. In contrast, the mammary tissue provides a more challenging environment which selects heavily for isolates that can survive the specific immunological responses as shown by Pryor *et al* [20]. The significance of our management of the dairy herd is that we inadvertently provide the heavily selected, 'cow adapted' isolates with opportunities to infect other cows via the vector of the milking machine or milking technicians' hands. Whilst less common now, some herds may still be exposing the developing gastrointestinal tract of herd replacement heifer calves to these 'cow adapted' 'potentially contagious' *S. uberis* isolates when they feed high somatic cell count milk or antibiotic treated quarter milk to those calves. It is unclear if the practice is significant or if other routes of exposure of potentially high levels of heavily selected 'contagious' *S. uberis* isolates to a wider environmental population are important, such as through discarding mastitic milk into the slurry system for spreading on pasture. It has been shown that pasture contamination levels of *S. uberis* are likely to be related to faecal loading [16] and it is possible that exposure of the wider environmental *S. uberis* population to 'contagious', 'cow adapted' isolates may allow dissemination of the genetic elements which confer that trait and thereby increase the likelihood that new environmental infections will then translate into additional contagiously acquired infections. In this context a comparison with the proliferation of antibiotic resistance genes is appropriate because some of the same mechanisms apply and in the same commercial dairy setting. Shedding of antibiotic-resistant *E. coli* has been demonstrated to be higher in calves fed waste milk from clinical mastitis cases compared to those fed milk replacer and this shedding was shown to persist post-weaning [5]. Antibiotic resistance has been shown amongst *E. coli* cultured from slurry systems and associated with previous mastitis antibiotic treatment practices [14] and mathematical modelling of gene transfer in this slurry system suggested that for a pathogen such as *S. uberis*, with a high gene transfer rate, the propagation of antimicrobial resistance would be rapid and difficult to control [30].

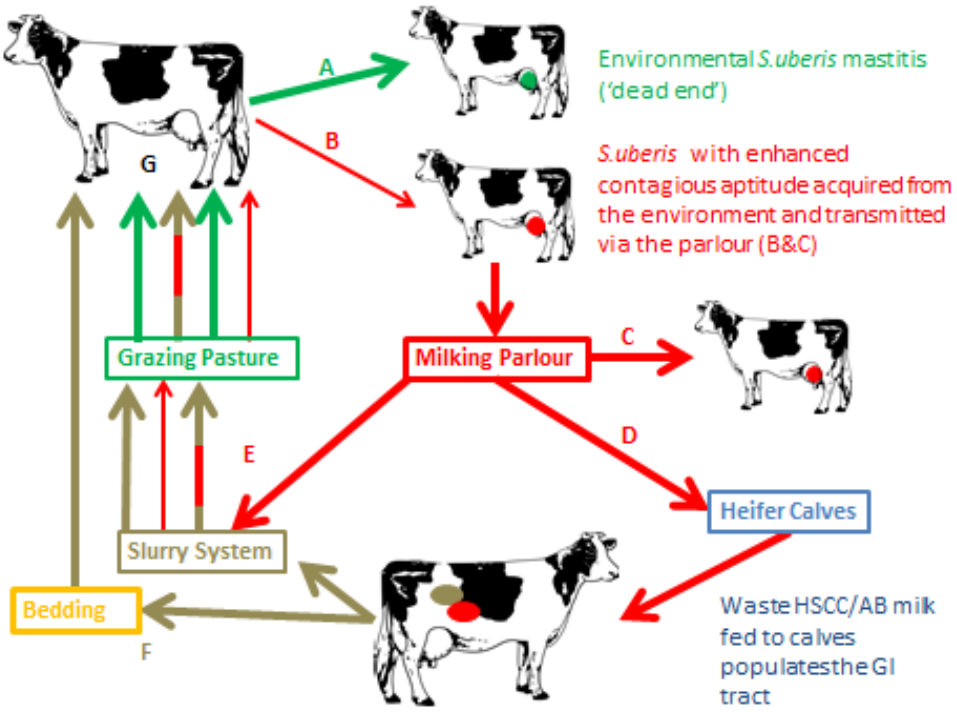


**Figure 4. Diagram of a potential *S. uberis* isolate/gene flow in a dairy herd. A - 'Environmental' origin mastitis case with no onward transmission. B - Initial infection from environment with an isolate of contagious potential. C - Contagious transmission via the milking parlour. D - Waste milk containing 'Contagious' *S. uberis* fed to herd replacement heifer calves, establishing gut flora and recombination opportunities. E - Waste milk containing 'Contagious' *S. uberis* and faeces with diverse Streptococcal spp mix in slurry system. F - Cows with gut flora containing 'Contagious' *S. uberis* isolates contaminate bedding. G - range of environmental infection sources (Bedding/pasture) with varying degrees of exposure to 'Contagious' *S. uberis*.**

This theory of management practices influencing the movement and propagation of bacterial virulence mechanisms through a population could explain the wide variation in herd mastitis patterns observed in this study, and differences from other counties - in particular New Zealand where very different pasture and slurry management is practised.

Adoption of automatic miking systems also poses interesting opportunities for bacteria to exploit different limitations in automated mastitis detection systems, as well as more opportunities for contact between cows as a result of more frequent milkings.

The predominance of a small number of similar sequence types across a large number of herds suggests that the virulence determinants for contagious transmission are dependent in some way upon specific features of this group. These may be epigenetic regulatory mechanisms, rather than specific virulence genes, acting in a way that enhances infectivity or immune evasion. Such mechanisms could act upon mobile genetic elements such as 'Clustered regularly-interspaced short palindromic repeats' (CRISPR) [13] that are otherwise unremarkable but when matched with the appropriate epigenetic manipulation, triggered possibly by external stimuli such as an immune response, they enable the bacterium to survive and replicate sufficiently to increase the probability of contagious transmission.

**Categorising pathogen behaviour**

The research summarised in this paper, coupled with clinical experience, challenges the paradigm that mastitis pathogens can be categorised based purely on their species. It is not uncommon to see sporadic cases of *S. agalactiae* and *S. aureus* with no evidence of spread to other cows or concomitant increases in bulk milk somatic cell count (BMSCC) or the prevalence of chronically infected cows. Likewise, as outlined above contagious behaviour of what were once considered to be environmental pathogens is now accepted.

In the absence of widespread availability of sub-species strain typing at a commercial level, practitioners need to rely on the clinical mastitis and somatic cell count (SCC) patterns in an attempt to quantify the relative importance of contagiousness in mastitis outbreaks. Useful parameters in assessing potential contagiousness in mastitis at a herd level include, but are not limited to, clinical mastitis recurrence and temporal distribution, lactation new infection rate, the prevalence of infection, duration of infection as measured by chronic infection rates, and dry period cure rates.

A variety of tools are available to help practitioners better understand the likely modes of mastitis transmission on farm; one such tool is the Mastitis Pattern Analysis Report freely available to farmers in the UK (https://ahdb.org.uk/knowledge-library/mastitis-pattern-analysis-tool).

Examples of different herd patterns illustrating varying degrees of contagiousness will be presented at the conference.

**Future opportunities and challenges**

The development in molecular genetic techniques over the past decade has been rapid, reducing costs, complexity and time as well as streamlining analytic processes. It is not unreasonable to imagine that in the foreseeable future the

discriminatory power which we can at present only achieve using research tools, will be deliverable by diagnostic laboratories at a price and timescale which is useful and attractive for dairy farmers and vets. This could reduce the uncertainty associated with determining the epidemiology of a mastitis outbreak and, used in combination with SCC data-driven approaches to mastitis pattern recognition, help with more targeted, cow specific decision making.

## CONCLUSIONS

Contagiousness in mastitis is a battle that needs to be continually fought because it offers such profound evolutionary survival/propagation advantages for pathogens. It will always be a threat even in herds that predominantly deal with environmental origin cases. A combination of laboratory techniques, and analysis of epidemiological patterns, can be used to identify the importance of "contagiousness" in a particular herd at a particular point in time. With time, the more sophisticated discriminatory methods currently used in research, may become commercially available. Understanding the contribution of contagious transmission in an individual herd is vital for targeting control measures.

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# ASSOCIATION BETWEEN GENOMICS AND MASTITIS

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## SUMMARY

The combined impact of sustained genetic improvement in the UK national dairy herd alongside enhancements to the management of udder health are reflected in an impressive, improved performance of UK cows. Levels of Somatic Cell Count (SCC) peaked in 2008, and annual improvements (reduction) show no signs of slowing yet. For mastitis, the disease incidence also continued to decline since 2009 as an indirect result of genetic selection for SCC. The availability of a direct mastitis evaluations since 2017 should aid gain for this trait, but here some attention is needed.

The more recent availability of genomic evaluations for both SCC and Mastitis will assist dairy producers to select with more precision for both of these traits and a considered breeding strategy should form part of any herd's health planning approach.

## INTRODUCTION

Dairy producers have done a remarkable job of breeding better cattle over the past 20 years, seen through improvements in virtually every trait for which there's a breeding index.

This is very evident in udder health traits which every producer is aware can have a profound effect on milk price and the profitability of a herd. And whilst many factors have been identified as contributing to improvements in SCC and mastitis, better genetics has unquestionably played a part.

UK milk recording organisations (CIS, Dale Farm, NMR, QMMS) started to collect SCC data routinely through the monthly milk recording from 1990 onwards. Since 1998, the UK has been using this national data to calculate SCC Predicted Transmitting Abilities (PTAs) as part of AHDB's genetic evaluation service (3).

SCC PTAs in the UK are expressed as a percentage and generally fall within the range +30 to -30. For every 1% in a bull's SCC PTA, a change of 1% in his daughters' SCC is predicted. For example, daughters of a bull with a -10% SCC are expected to have cell counts 10% lower than daughters of a bull with a SCC

PTA of zero. So, negative figures for SCC PTAs are desirable as these indicate a reduction in cell counts.

Since April 2017, Mastitis genetic evaluations have also been published for all dairy breeds evaluated in the UK (4). Expressed on a scale of about -4 to +4, daughters of a poor Mastitis PTA bull (+3) have about twice the chance of getting mastitis as daughters of sires with a favourable index (-3).

The availability of this genetic index, which has also been incorporated into the national profit index used for selection (Profitable Lifetime Index, £PLI) allows farmers to breed cows directly using a genetic selection tool aimed at improved resistance to mastitis.

Although there is a strong genetic link between lower SCC and a reduction in mastitis cases, there is a small number of bulls who reduce SCC but not necessarily reduce cases of mastitis. The benefit of a mastitis trait helps to identify those bulls and allow farmers to make more informed breeding decisions for their herd.

Genomic evaluations for mastitis resistance are also available from AHDB for the Holstein breed. This genomic prediction is based on close to 27,000 genotyped Holstein sires with daughter information on mastitis. These bulls either have daughters milking in the UK (approx.10,000), or have daughters recorded for mastitis information in other countries which is converted to UK equivalents by the International Bull evaluation service (INTERBULL).

These genomic predictions can be used to predict the genetic merit of young animals without any performance recording themselves (i.e. young bulls or heifer calves). With the increased uptake of genomic testing of females, this enhanced genomic insight further improves on the ability to fine tune our genetic selection.

This paper demonstrates the improvements in genetic indexes for both SCC and mastitis across the national herd and highlights how genetics and genomics can help producers to improve the observed phenotypes for udder health.

## MATERIALS & METHODS

Data from lactations 1 to 5 are used for the genetic evaluations of SCC and mastitis in the UK. These data are extracted from the milk recording databases and undergo validation before being joined to ancestry information. The heritability of both these udder health traits are low, but with large amounts of data available for modelling, accurate genetic estimates can be derived. The heritability of SCC is 0.11 and for mastitis 0.04 (1).

For this analysis a subset of these validated data was used (April 2025) for cows calving between 2003 and 2023. Data was restricted to Holstein cows who had their sire and dam recorded and whose parents were also evaluated. These cows needed to have a valid mastitis record and calved in herds which had at least 10 cows calving per selected year and lactation, and who further reported at least 4% incidence of mastitis in that particular year-lactation class. These restrictions were introduced to try to exclude herds with limited recording of ancestry or mastitis events. Through this paper the incidence is a metric of having at least one case of mastitis during a lactation (0=healthy, 1=clinical case recorded). The final dataset used in this analysis contained 2,623,973 lactation records.

Note that the dataset excludes lactations greater than five. Given that the incidence of mastitis rises with lactations, this means the trait averages reported in this paper will be slightly lower that those more typically referenced. However, this does not affect the trends observed or the ability to illustrate the impact of genetics on performance.

To demonstrate the additional value of genomic testing, a second filter was applied which restricted it to cows which were genomically tested and calved in the five-year period of 2019 to 2023. The more recent years were chosen as genomic testing of cows was limited in the first few years when genomics was first made available in the UK (2012), and these cows needed to have sufficient time to complete five lactations used in this analysis. A total of 51,550 records were used in this second analysis.

## RESULTS

### Impact of genetic indexes

The availability of SCC PTAs alongside farm management improvements has contributed significantly to the long-term industry reduction of SCC levels. Figure 1 plots the average genetic merit of the cows used in the analyses against their average geometric SCC yields (x1000) taken from lactations 1 to 5. These trends show that the average genetic merit of SCC started to improve from 2008, which coincided with the improvements seen in their phenotypic performance. The genetic merit of cows calving in 2023 averaged -2 (PTA SCC) and is not showing signs of slowing down. For reference, the average PTA SCC for Holstein calves born in 2024 averaged -6, and the average genetic merit of service sires used in 2024 was -10 (source: AHDB). This implies that under normal circumstances the national herd will continue to benefit from these genetic gains already made and will continue to show reduced SCC yields in the coming years.

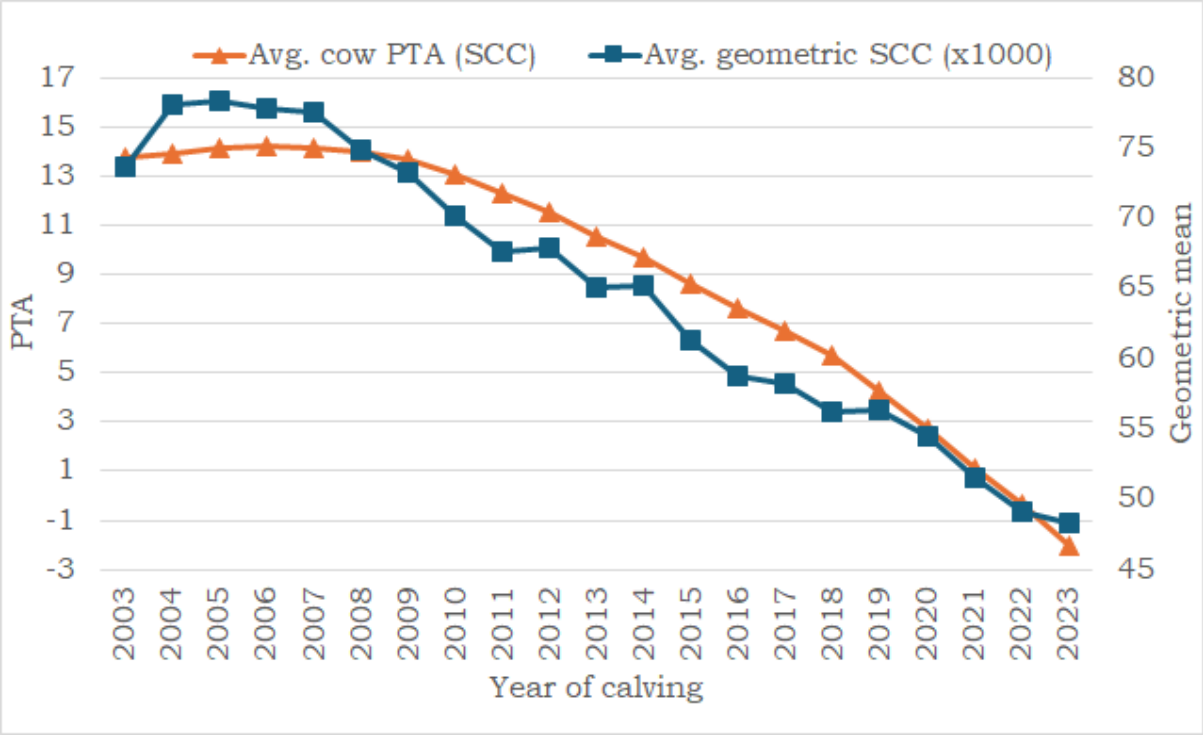


**Figure 1. Trends by year of calving for the average cow SCC PTA and their corresponding average geometric SCC yield (x1000) in lactations 1 to 5.**

The improvement in genetic merit of SCC indirectly also improved the genetic merit for mastitis, due to a 0.7 genetic correlation between these two traits. These gains contribute to the improvement of the incidence of mastitis observed. Figure 2 shows a similar trend to those seen for SCC (figure 1), but for mastitis the peak genetic merit came a year later in 2009 and has seen a gradual improvement since. Similarly, the phenotypic performance of these cows in their first five lactations has seen a gradual improvement from about the same time. Like SCC, the genetic merit of future generations is expected to continue to improve, but gains are slowing down compared to the near linear improvement seen in SCC. Cows calving in 2023 average -0.1 mastitis PTA. This compares to an average genetic merit of Holstein calves born in 2024 of -0.2, and the average service sire used has a PTA Mastitis of -0.5. These smaller genetic gains are likely to be reflected in much smaller improvement of the mastitis incidence in future years compared to what we have observed in the last decade.

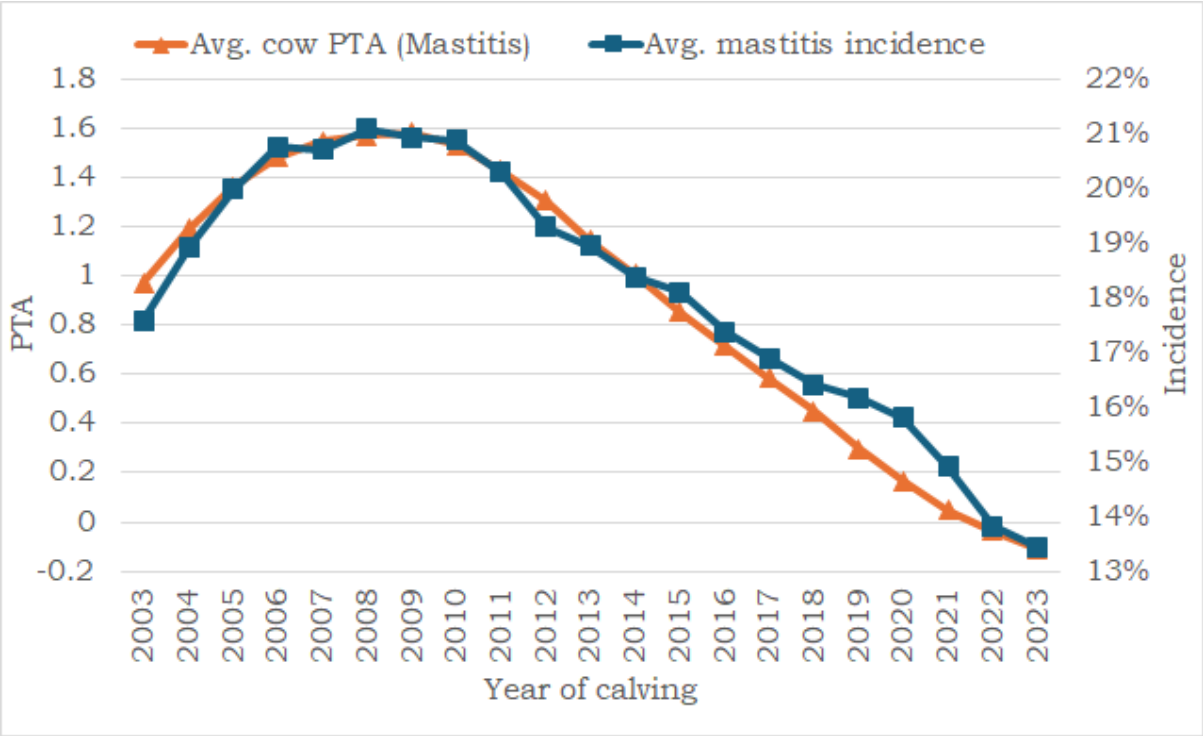


**Figure 2. Trends by year of calving for the average cow mastitis PTA and their average incidence of mastitis in lactations 1 to 5.**

Splitting the data used in Figure 2 into separate lactations reveals that cows calving for their first lactation show no gains in PTA mastitis since 2021 and are also showing signs of a halt in improvement of their phenotypic performance (figure 3). Because cows calving in lactation 2 to 5 continued to improve, due to the lag of genetic gains made in earlier years trickling through to the older generations, the overall average shows the gains continuing as observed in figure 2.

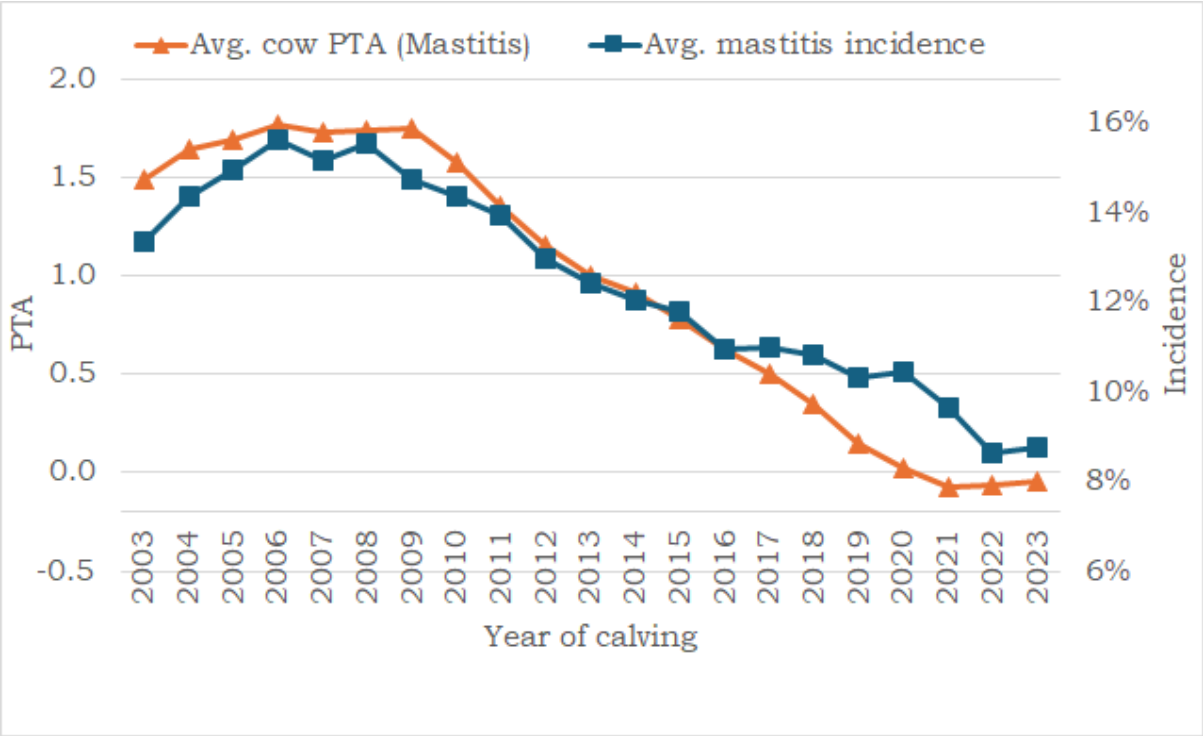


**Figure 3. Trends by year of calving for the average cow mastitis PTA and their average incidence of mastitis in lactation 1 only.**

**Value of genomic testing**

The use of genomic young sires for breeding is now common practice (>75% of Holstein inseminations in 2024. Source; AHDB). However, the uptake of genomic testing of females has been slower. Never-the-less 27% of all 2023 born Holstein calves in milk recorded herds have been genomically tested and each year this proportion continues to grow. This new insight, combined with the high usage of sexed dairy semen gives the dairy producer new tools to continue to make gains for the important udder health traits (SCC, Mastitis).

The PTA values for individual traits are primarily calculated to identify animals that can produce the best future progeny (as implied by the name Predicted Transmitting Ability). But as well as being used to select animals to breed from, the genetic merit of these animals also correlates to their progeny's future performance, or indeed an animal's own performance.

To illustrate the relative benefits of using different selection strategies, the second smaller data set of genomically tested females was analysed and is presented in figures 4 and 5 below. Figure 4 first gives the relationship between the average genetic merit of a cow's sire, and her own performance. In other words, how well does the progeny of good sires (PTA < 0) perform compared to

poor sires (PTA > 0). Note; this is typically the main strategy used by farmers to breed for a better performing herd.

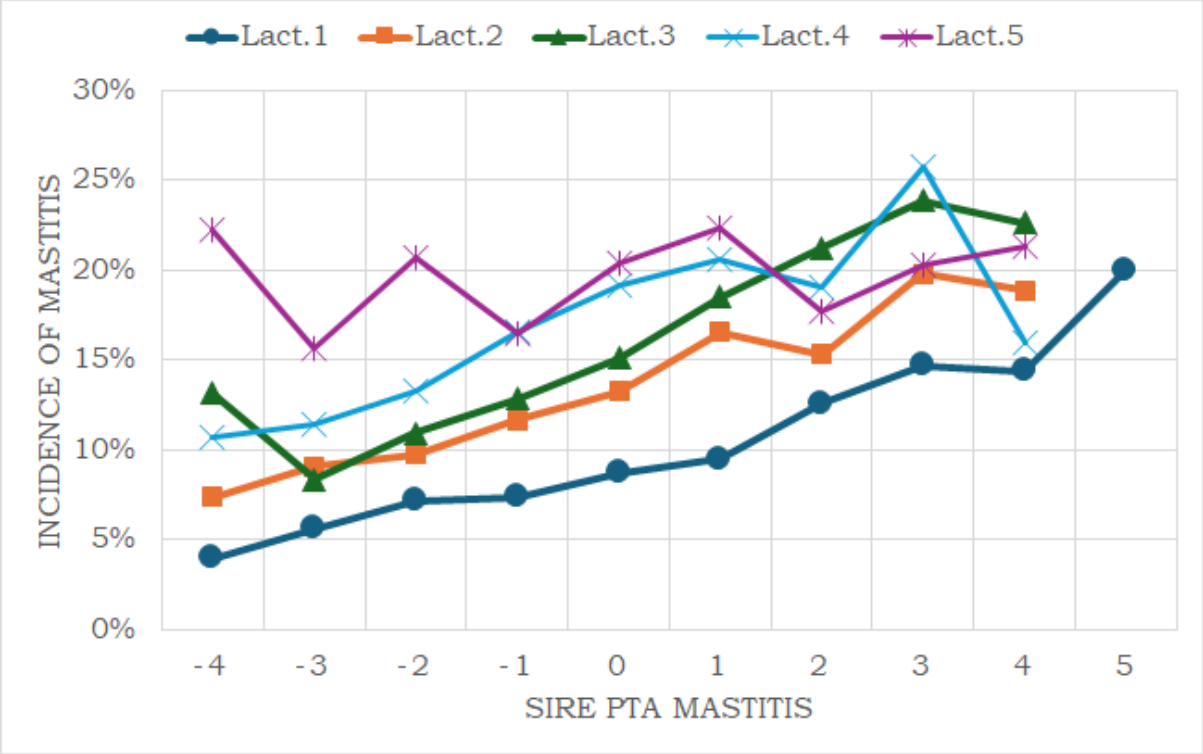


**Figure 4. Relationship between the sire's mastitis PTA and average incidence of mastitis for its progeny in lactations 1 to 5.**

The data in figure 4 shows that, as expected, the average incidence of mastitis increases with lactation but also illustrates the degree with which the incidence rises as the sire's PTA for mastitis gets worse (i.e. gets higher). Note that the relationship gets stronger as animals get older (i.e. the effect of genetics on the performance is more pronounced. Lactation 5 however shows a level trend, this is partially because the number of animals is fewer and therefore a less precise measure, but is also a reflection of the fact that the graph depicts the expected progeny's genetic merit, and bad performing progeny (for whatever reason) will have left the herd by the time the progeny makes it to lactation 5.

The slope of lactation 1 averages is 1.1% (i.e. every 1 point increase in a sire's PTA results in 1.1% higher incidence of mastitis in lactation 1), 1.4% for lactation 2 and 2.0% for lactation 3.

Figure 5 below adds an additional piece of information as these are now based on the cows own genomic prediction. The genomic prediction used in the analysis deliberately ignores the animal's own performance and is purely based on its

genotype value to avoid bias. The graph shows a similar picture, but in this case the prediction accuracy improves, and the slope of the lines similarly increases. The regression of the genomic prediction on performance is 2.1% in lactation 1 (i.e. every point PTA mastitis for the cow results in a 2.1% change in incidence), 3.4% in lactation 2 and 3.9% in lactation 3.

Note in particular how lactation five data does not show a levelling off as it did for sires but continues to contribute to the cow's performance. In this case the good genetics (<0 PTA) manage to keep disease incidence as a low level, whereas the poor genetics are at a much higher risk of getting clinical mastitis.

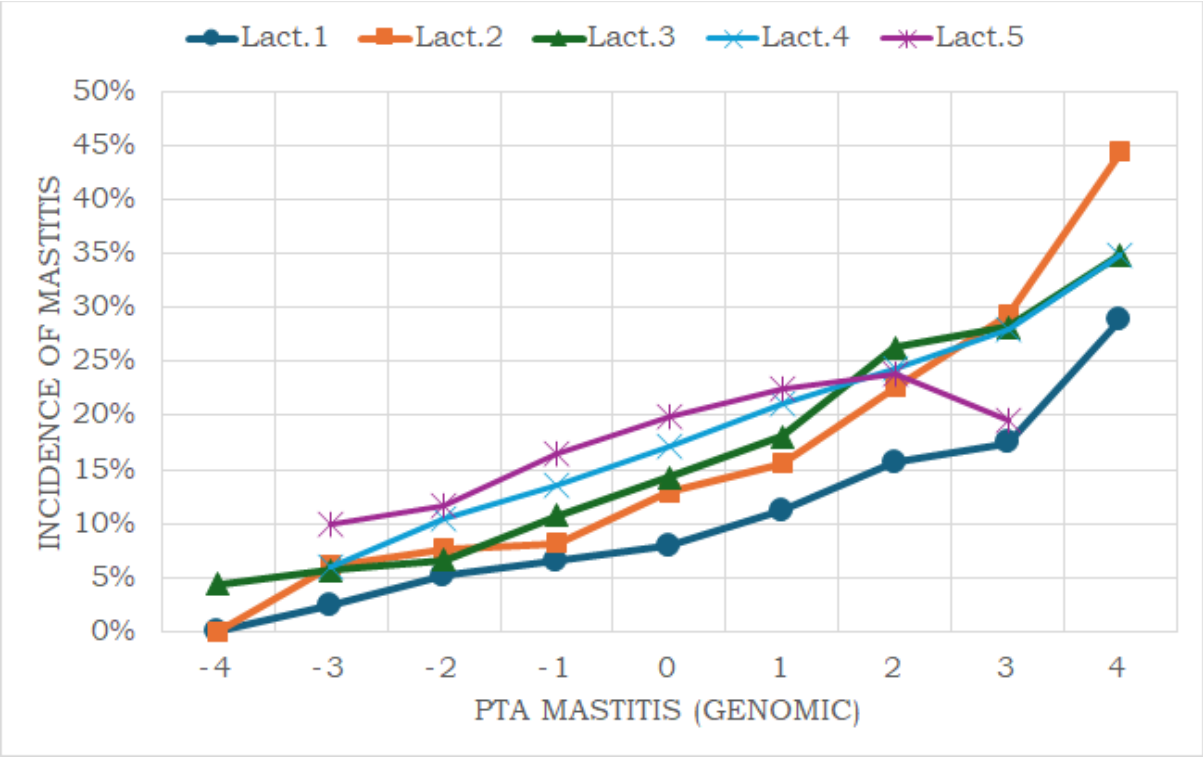


**Figure 5. Relationship between the genomic mastitis PTA for cows and their average incidence of mastitis for lactations 1 to 5.**

## DISCUSSION

The use and impact of SCC PTA's was previously described in the 2008 paper presented at this conference, in which the prediction was made that based on the genetic trends observed for SCC, the national phenotypic levels of SCC's would peak in 2008 and show a decline from there (5). These predictions have shown to be remarkably correct and national average SCC's reported in 2008 saw indeed the highest levels peaking at 197,000 and have since shown a steady

decline to 155,000 in 2024 (https://ahdb.org.uk/dairy/gb-milk-hygiene accessed May'25).

NMR's latest Key Performance Indicator (KPI) report (2) – in which 500 herds of Holstein Friesians are used as a representative cross-section of the national herd – demonstrates this well.

This shows that in 2023, 70% of herds kept average cell counts below 200,000 cells/ml, compared with only 44% in 2010. Also in 2023, more than half (52%) of all cows in the 500-herd sample completed their lactations without recording an SCC above 200,000 cells/ml. This compares with 2010, when only 35% of cows avoided this high cell count figure.

Mastitis incidence similarly declined, and respondents within the 500-cow study saw an average of 22 cases per 100 cows per year in 2023, down from 36 cases per 100 cows in 2016.

This paper similarly showed that the incidence of mastitis has already peaked in 2009 and continues to see improvements year on year. But the data also revealed that care needs to be taken for genetic selection on mastitis PTAs as these performance gains may not be achieved as easily going forward.

It is worth remembering that genetic selection is a relatively cheap and permanent approach to achieve performance gains compared to other management changes.

And although improving genetics is never a quick fix, once embedded in a herd, genetic improvement will persist and accumulate over the generations.
It also chimes with all of the farming industry's efforts as we are breeding animals which are innately easier to manage and require fewer antibiotic interventions.

Scope for further improvement over those already achieved today remains.
These gains can either come from the choice of dairy sires available to producers, but importantly also through greater uptake of genomic testing of females to aid targeted selection and breeding decisions. As with any trait, the management of individual cows will impact their performance (1), but having knowledge of the genetic quality of the calves that are being reared or used to breed the next generation will greatly increase the odds of a favourable outcome.

Producers aiming to improve udder health through better genetics are advised to consider the following five options within a broader breeding strategy.

1. The first and perhaps most obvious and easy to implement is the use of the Mastitis PTA of service sires used in the herd. It is advised to only use bulls

with negative PTAs to reduce the incidence of the disease and in particular bulls with above zero mastitis PTAs should be avoided.

2. A similar pattern exists for SCC PTAs, which is the other PTA which should be a priority when seeking improvements to udder health. As with Mastitis, bulls with a negative score will reduce cell counts. Bear in mind that although there is a strong link between SCC PTAs and Mastitis PTAs, a small number of bulls will reduce SCC but won't necessarily reduce cases of mastitis. The Mastitis PTA helps to identify these bulls.

3. Milking speed gets a mention as a trait for genetic selection as some producers are concerned that bulls with the best cell count scores may also slow down milking. However, correlations between ease of milking and udder health traits are low, confirmed at 0.09 in a recent AHDB analysis (unpublished), which showed the relationship between genetic index for milking speed and observed incidence of mastitis. The evidence is therefore clear, that selection in favour of udder health has not slowed down milking. However, producers naturally want to avoid extremes for this trait for management reasons, and if they have concerns, they can check the ease of milking score of any bull being considered. This is expressed on a scale of about -3 (slow) to +3 (fast).

4. Udder conformation has been part of selection for many years for a range of reasons, and udder depth and fore udder attachment in particular have a small association with the incidence of mastitis. However, correlations are considered low, particularly when compared to direct selection for Mastitis or SCC Index. This means that as expected, the direct udder health indexes have a far higher association with actual cases of mastitis observed in progeny than udder conformation traits. For this reason, the advice is always to prioritise Mastitis and SCC PTAs for genetic selection if seeking to improve udder health. As with all genetic selection, it's always better to use the PTA for the trait you seek to improve, rather than a proxy.

5. Finally, the Healthy Cow index (£HC) was introduced in 2021 and is helping dairy producers identify the best bulls for improving all aspects of herd health (https://ahdb.org.uk/knowledge-library/healthycow-index).
However, within this composite index, 23% is assigned to udder health, meaning producers who refer to £HC will not only improve overall health – and reduce the cost of poor health – but will specifically improve SCC and mastitis. Such composite indexes are particularly valued by producers who favour simplified genetic selection, as £HC is an index which gives an at-a-glance picture of a bull's ability to transmit good overall health.

The above five-point strategy highlights the importance of sire selection, but these points extend to the choice of cows to breed from. Improving both sire and

dam choices, aided by genomic insight, can greatly accelerate genetic progress being made.

For any dairy producer, udder health should be amongst the top considerations for genetic improvement. Therefore, it is strongly recommended to have a considered breeding policy as part of a herd's health planning approach.

## CONCLUSIONS

Improvements in udder health have been continuing and significant for nearly two decades now, and the genetic trends suggests this will continue to be the case in future years. Selection for mastitis however requires attention to ensure the industry can improve for this trait at the same rate.

Luckily the genetic merit of sires available for use in the UK offers plenty of opportunities to improve on both these traits, and the availability of genomic testing for females offers new and improved ways to make more informed, and potentially more rapid genetic gains than was achievable in the past.

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# CHLORINE DIOXIDE FARM WATER TREATMENT REDUCES MASTITIS RATE

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## SUMMARY

Water contamination represents a potential risk factor for mastitis and udder health challenges. The presence of pathogens in farm water from incoming supplies, potentiated by biofilm and pathogen growth within on-farm water infrastructure can be linked to raised environmental mastitis cases. Chlorine dioxide is a powerful, but safe, oxidising agent and biocide.

A chlorine dioxide water treatment system was added to a dairy farm, and udder health parameters compared between the 12 months prior to installation, and the 12 months after installation.

The clinical mastitis rate saw a 37% reduction, with further reductions in bulk tank somatic cell count, bulk tank bactoscan, and the proportion of readings for these parameters above a threshold, all of which were statistically significant. Chlorine dioxide water treatment may be an important tool in the reduction of udder health challenges on dairy farms.

## INTRODUCTION

Water is fundamental to the health and productivity of dairy cattle, serving not only as a vital nutrient for hydration and digestion but also as a potential vector for pathogenic organisms. In dairy operations, the quality of water used for herd drinking, parlour wash down, udder hygiene, and equipment cleaning may influence udder health.

Contaminated water has the potential to harbour a variety of microorganisms that, when introduced to the teat canal during milking, can trigger intramammary infections, leading to both clinical and subclinical mastitis [1,4]. These pathogens can exist in the water supply, especially where private or alternative water supplies exist, replicate rapidly within the pipework infrastructure on a farm and contaminate the water supply through faecal spread [2]. Consequently, improving water hygiene should be considered as a critical component of effective udder health management strategies on modern dairy farms.

Chlorine dioxide ($ClO_2$) has emerged as a promising agent for the disinfection of farm water due to its exceptional oxidizing properties – it has an Oxidation-

Reduction Potential (ORP) of 600-1000mV, whilst maintaining residual activity whilst in solution in water [3]. Unlike traditional chlorine-based disinfectants, $ClO_2$ disrupts microbial cellular structures by oxidizing key biomolecules such as proteins and lipids, thereby rendering pathogens inactive without forming significant levels of toxic by-products. Its efficacy across a broad pH range and its ability to penetrate microbial biofilms further underscore its suitability for treating water supplies used in livestock environments. By effectively reducing the microbial load in water, chlorine dioxide has the potential to decrease the incidence of both clinical and subclinical mastitis, ultimately contributing to improved udder health and higher milk quality.

## MATERIALS AND METHOD

A trial farm was selected milking approximately 260 cows. The farm is supplied by 2 separate boreholes, with mains water used as a back-up. The longitudinal study will compare udder health parameters for the 12 months prior to treating the incoming farm water with $ClO_2$ (PRE), and 12 months with treatment (POST). A 12 month period was selected to mitigate for any seasonal effects. Prior to treating the water, all incoming borehole water flowed past a UV bulb.

The $ClO_2$ treatment system involved a pressure-driven $ClO_2$ production process for high efficiency of production, with controlled delivery directly into the water feed. An initial period of higher-dose $ClO_2$ is used to oxidise any biofilm or bacteria within the pipework and troughs, before titrating $ClO_2$ dose rates to target 0.5 parts per million at all points in the water supply. The initial period was excluded from the data collection period.

The udder health parameters studied were all collected as part of the farm routine – Clinical Mastitis Rate in cases per 100 cows per year (CMR), bulk tank Somatic Cell Count (BTSCC), proportion of BTSCC over 100,000 cells/ml (BTSCC>100), bulk tank Bactoscan (BTB), proportion of BTB over 50,000/ml (BTB>50), individual animal Somatic Cell Count (ISCC), and proportion of ISCC readings over 200,000 cells/ml (ISCC>200). The farm has excellent records, and reported no other changes relating to udder health management.

## RESULTS

The parameters recorded for both the PRE and POST periods were collated and analysed as per Table 1. P values were calculated using excel with T-tests performed for BTSCC and BTB, and Chi square tests for other parameters.

In the 12 months with water treated by $ClO_2$, the herd saw a 37% reduction in CMR from 27 cases per 100 cows per year to 17 cases per 100 cows per year. BTSCC also saw a 28% reduction between the 2 periods, with a 69% reduction in the proportion of bulk tank recordings with readings over 100,000 cells/ml.

The BTB saw an 81% reduction, with a 71% reduction in the proportion of bactoscan readings over 50,000/ml. These were all significant reductions, with p values less than 0.05.
There was no significant change in the proportion of individual cow readings with SCC over 200,000 cells/ml.

**Table 1 Comparison between dipping and spraying**

| Parameter | Pre | Post | % Change | p value |
|---|---|---|---|---|
| Cow Numbers | 264 | 252 | -5% | |
| Mastitis Rate/100 cows/year | 27 | 17 | -37% | <0.01 |
| Bulk tank SCC, '000 cells/ml | 119 | 86 | -28% | <0.01 |
| Bulk tank % > 100,000 cells/ml | 72% | 22% | -69% | <0.01 |
| Bulk tank Bactoscan, '000/ml | 86 | 16 | -81% | 0.03 |
| Bulk tank Bactoscan % >50,000/ml | 24% | 7% | -71% | 0.02 |
| Proportion ISCC >200,000 cells/ml | 11% | 9% | -18% | 0.52 |

## DISCUSSION

On this farm, the addition of a $ClO_2$ water treatment system was associated with significant reductions in most udder health parameters, most notably Clinical Mastitis Rate, bulk tank Somatic Cell Count, and bulk tank Bactoscan, with the bulk tank assessments also having a smaller proportion of readings above specific thresholds. The $ClO_2$ system will not only kill pathogens present in the incoming water feed, but clear biofilm and pathogens from pipework and troughs on the farm.

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# UTILISATION OF MULTIPLE SAMPLE PROTOCOLS TO FACILITATE GENOCELLS® SOMATIC CELL COUNT MEASUREMENT IN LARGE HERD SCENARIOS

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## INTRODUCTION

Streamlined measurement of somatic cell counts (SCC) are the goal of many new technologies due to the benefit of more regular testing in mitigating the economic losses associated with lower milk quality and animal health, combined with reducing the inconvenience of individual animal sampling. An established route to simpler SCC measurement, through analysis of bulk milk samples, was established through research [1] and subsequent commercial development of GenoCells® [2] in France.

To perform GenoCells, a homogeneous aggregate milk sample is required along with yield and identification of all animals included in the sample. All contributing animals must be genotyped and the technology then uses a genotype analysis of the milk sample to calculate each animal's contribution to the DNA load of the sample (which is her percentage contribution to the overall somatic cell load of the sample). Utilising the yield data alongside a datum SCC measurement of the aggregate milk sample allows conversion of this result into individual animal SCC measurements.

The service is well established in France but the herd demographic is different to that in the UK with many herds less than150 cows. An established principle of this technique is that, as numbers of animals contributing to the sample increase, then DNA dilution increases and overall reliability of the resulting SCC decreases[1] unless higher density SNP arrays or sequencing is used. These alternative testing options are not yet economically viable for a commercial SCC management service.

However, the advantages of simple in-parlour routines to measure SCC become more significant as herd size increases. Therefore, alternative solutions are required to mitigate the effects of DNA dilution as herd size increases. Similarly, the ability to collect good quality, homogeneous samples where farms have silos or pump directly to a road liner also present problems both for GenoCells but also other applications where representative milk samples are required.

This study examined the commercial opportunity to utilise GenoCells technology on a "divide and rule" basis, that is taking several samples during milking by

collecting from subgroups of cows rather than one overall sample at the end of milking. Such a technique would mitigate the effects on result quality caused by increasing herd size as it allows the technique to be applied to multiple samples, each containing an optimal or convenient number of cows per sample.

Previous exploratory work in the US completed by National Milk Records indicates that this provides very reliable results in systems where there is a continuous, flat rate milk flow from the parlour to the farm vat however these systems are not common in the UK. This study was based upon trialling a novel method of sampling in standard UK parlour systems where the parlour milk pump runs periodically with operation controlled by the level of milk in a receiver vessel (level control systems). The aim was to test the principle that this novel approach could produce suitable quality samples for both GenoCells and for other applications requiring string sampling in these systems. It should be noted that at the time of publication, the trial was ongoing so these are preliminary results and will form part of a larger SRUC study on applications of GenoCells.

## MATERIALS AND METHODS

A farm was selected with over 600 in-milk animals split across multiple groups in a standard AYR high input, high output, three times milking system. The animals were herringbone milked, meaning that clear points of differentiation between groups could be identified, this provided opportunities to complete sampling of one group and commence sampling of the next with clear delineation of milk samples. The data contained an exact list of contributors and yields for each animal, all cows were genotyped prior to the trial commencing

The milk was line sampled using the NMR prototype sampling device in conjunction with a Watson Marlow 120F peristaltic pump. The parlour milk pump operated on demand and ran at a constant flow when operational. The parlour milk line was sampled between the pump and the filter.

The trials were completed on standard test day weighing sessions to provide accurate comparative data. The aggregate milk samples were uniquely identified and tested on Illumina 56k array plus FOSS analysis to provide all data required for the calculation of individual SCC. All individual samples were submitted for FOSS analysis. The technology was then applied to generate the SCC for comparison to individual cow results.

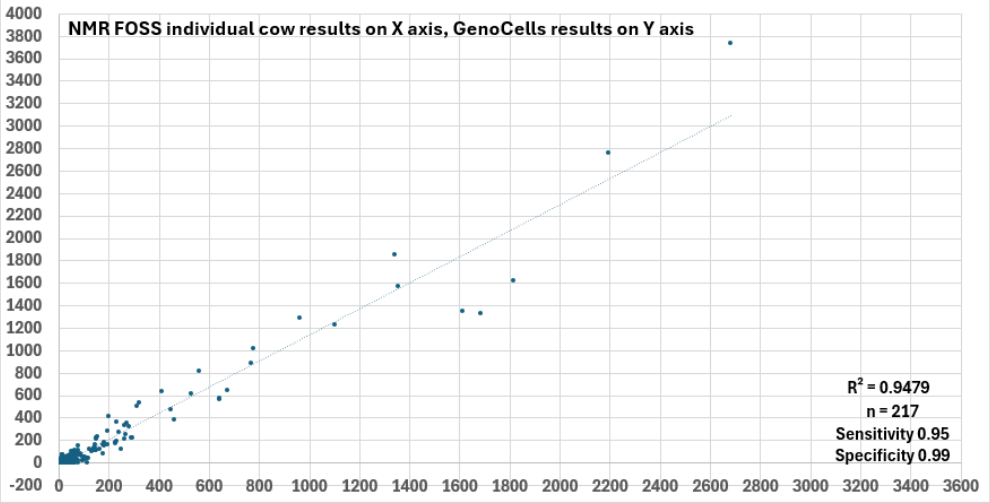
## RESULTS

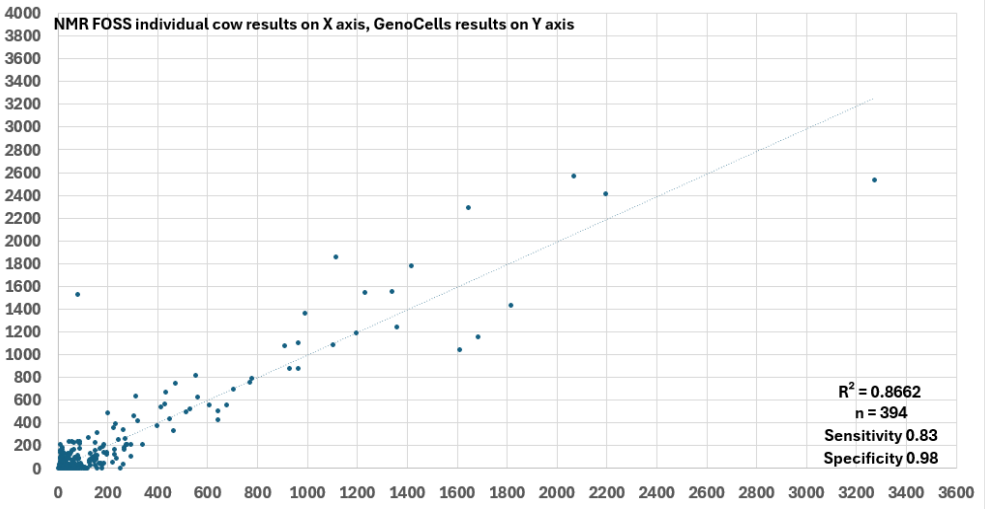
Results are shown for two of the line samples, one containing 217 cows and the other containing 394 cows. The changes in reliability associated with DNA dilution can be seen in the differences of sensitivity and specificity as well as R

squared. These are in line with the expected results for GenoCells outlined in the research.

The results indicate that the novel technique applied for line sampling from level control systems can provide a viable and suitably accurate method for obtaining representative, homogeneous samples of milk for GenoCells and other applications. This technology will allow larger herds to complete GenoCells services by taking a small number of line samples during milking at intervals suitable to the requirements of the service. One aim of the full study is to identify the number of cows that can be contained in a milk sample to achieve a specific SCC threshold at a required level of reliability.

The results below show comparative results of FOSS individual animal SCC results and GenoCells line sampled aggregate sample derived SCC.



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# FACTORS ASSOCIATED WITH BULK TANK SCC ON IRISH DAIRY FARMS

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## SUMMARY

- Lower bulk tank somatic cell count ($log_{10}$BTSCC) was associated with seasonal calving patterns, family involvement in milking, mastitis treatment records, comprehensive pre-milking preparation, and post-milking teat disinfection.
- Higher $log_{10}$BTSCC was linked to longer morning milking durations and certain parlour designs, including rotary parlours and those with straight breast rails or backing gates in the collecting yard.
- Reduced $log_{10}$BTSCC was observed with the use of automatic cluster removers, automatic washers on the milking machine, and strategic selective dry cow therapy - especially when guided by multiple resources and veterinary input.

## INTRODUCTION

Bulk tank somatic cell count (BTSCC) is of one of the most important quality parameters of dairy milk production. Irish dairy herds have expanded significantly to meet rising demand for milk and dairy products. This growth poses challenges for individual animal monitoring, increases milking times, and places greater stress on milking techniques and mastitis control strategies. This is of particular importance in the advent of changes to veterinary medicine legislation Regulation (EU) 2019/6 and the requirement for farmers to be more prudent with their antimicrobial use. This research looked at factors which were associated with BTSCC on Irish dairy farms.

## METHODS

An online survey was distributed by milk processors in June 2022. Its purpose was to assess milking management practices and parlour facilities in Irish dairy herds. The survey comprised of 66 questions and investigated five main areas; farm-, parlour-, and milking-specific management, somatic cell count (SCC) control strategies and farmer-specific attitudes and behaviours [1].

Survey respondents were geographically distributed across the 4 provinces of the Republic of Ireland, with a total of 24 out of 26 counties represented. Of these, 222 respondents were from Munster, 33 from Connaught, 100 from Leinster and 21 from Ulster (376 total). Associations were drawn between the answers of each survey section and the farms' monthly logarithmic-10 transformed BTSCC ($log_{10}$BTSCC) from January 2021 to August 2022 in order to investigate which factors were significantly associated with increased or decreased BTSCC.

Five multivariable mixed-effects models were developed, each aligned with a key survey theme. $Log_{10}$BTSCC was the dependent variable. Fixed effects included month, year, and milk volume, with herd ID modelled as a repeated measure. Variables significant in univariate analysis ($p < 0.1$) were included in the initial models, and backward stepwise elimination was applied until all retained variables were significant at $p < 0.05$. Modelling was conducted using the PROC MIXED procedure in SAS OnDemand for Academics.

## RESULTS

Effect sizes (ES) and significance (p) values below are derived from the final mixed-effects models. Variables retained in each model represent the strongest associations identified after stepwise elimination. Results are grouped by thematic area.

### *Farm-specific associations*

Seasonal calving patterns were associated with a decreased $log_{10}$BTSCC compared to split calving herds (ES -0.08; p = 0.006). A combination of the respondent milking alongside a family member was associated with a decreased $log_{10}$BTSCC compared to an employee milking alone (ES -0.1; p = 0.029), an employee milking in conjunction with the respondent (ES -0.06; p = 0.01), the respondent milking alone (ES -0.04; p = 0.027), or a family member milking alone (ES -0.07; p = 0.042). Not keeping mastitis treatment records was associated with a significant increase in $log_{10}$BTSCC (ES +0.06; p = 0.005), as was a longer duration of morning milking (ES +0.0007; p = 0.004).

### *Parlour-specific associations*

Rotary parlours were associated with a significant increase in $log_{10}$BTSCC compared to herringbones with recording jars (ES +0.19; p = 0.0006), swing-over herringbones (ES +0.14; p = 0.001) and parallel parlours (ES +0.16; p = 0.008). The presence of automatic cluster removers (ES -0.03; p = 0.047) and automatic washers on the milking machine (ES -0.05; p = 0.009) were associated with a significant decrease in $log_{10}$BTSCC. The presence of straight breast rails (ES

+0.07; p = 0.023) and backing gates in the collecting yard (ES +0.05; p = 0.022) were associated with significantly increased $log_{10}$BTSCC. Farms which practiced cluster disinfection were associated with a significant decrease in $log_{10}$BTSCC compared to farms which did not (ES -0.04; p = 0.014).

***Milking-specific associations***

Never conducting foremilking was associated with a significant increase in $log_{10}$BTSCC compared to foremilking as part of a milking routine (ES +0.08; p = 0.027) or foremilking after calving (ES +0.14; p = 0.031). Foremilking on the suspicion of both clinical mastitis and subclinical mastitis combined was also associated with a significant increase in $log_{10}$BTSCC compared to foremilking as part of a milking routine (ES +0.07; p = 0.018) or foremilking after calving (ES +0.13; p = 0.035). No pre-milking udder preparation was associated with a significant increase in $log_{10}$BTSCC compared to a combination of a disinfection and drying step (ES +0.09; p = 0.0004). A combination of disinfection and drying was also associated with a significant decrease in $log_{10}$BTSCC compared to a drying step alone (ES -0.08; p = 0.002), a disinfection step alone (ES -0.13; p = <0.0001), a combined wash and drying step (ES -0.09; p = 0.011), and a combined wash and disinfection step (ES -0.19; p = 0.006). No post-milking teat disinfection was associated with a significant increase in $log_{10}$BTSCC compared to spraying (ES +0.10; p = 0.034) or automatic in-cluster dipping (ES +0.31; p = 0.007). Automatic in-cluster dipping was associated with a significant decrease in $log_{10}$BTSCC compared to spraying (ES -0.22; p = 0.042) or dipping (ES -0.24; p = 0.034).

***SCC control associations***

Decisions for selective dry cow therapy made on the basis of all available resources (i.e. individual cow factors and milk recordings, records of clinical cases and their outcomes throughout lactation, milk yield records and CMT testing), were associated with a significantly decreased $log_{10}$BTSCC compared to most other combinations of these resources. Conducting eleven milk recordings in 2021 was associated with a significant decrease in $log_{10}$BTSCC compared to conducting zero (ES -0.15; p = 0.013) or four (ES -0.15; p = 0.025) milk recordings.

***Farmer-specific associations***

Farmers dairying less than five years were associated with a significantly lower $log_{10}$BTSCC than those dairying greater than five years. Farmers who sought consultation with advisory services alone for SCC advice were associated with a significantly higher $log_{10}$BTSCC than those using advisory services in conjunction with their veterinary professional (ES +0.09; p = 0.001), a combination of their veterinary professional and peer-to-peer communication

(ES +0.09; p = <0.0001), and a combination of their veterinary professional and self-directed learning means such as magazines, websites or handbooks (ES +0.07; p = <0.0001).

## CONCLUSION

Lower $log_{10}$BTSCC was associated with a combination of hygienic milking routines, targeted use of automated parlour technologies, and mastitis incidence record-keeping. Strategic decision-making, especially when supported by veterinary input and milk recording data, played a critical role in effective BTSCC control across Irish dairy farms.

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# PRECISION VACUUM CONTROL IN CONVENTIONAL AND AUTOMATIC MILKING INSTALLATIONS

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This paper will describe studies performed on new vacuum control strategies Flow Responsive Milking™ (FRM) introduced by DeLaval for both conventional and automatic milking systems. One study was conducted on Flow Adjusted Vacuum (FAV) applied on a commercial rotary milking parlor. Conventional vacuum control (CON) maintained constant milkline 45 kPa vacuum during the entire milking process, while the FAV system applied milkline vacuum of 40 kPa milkline vacuum during the low flow period and 48 kPa when the milk flowrate of an individual cow exceeded 2 kg/min. Peak milk flowrate increased by 12%, average milk flowrate increased by 4%, and milking duration decreased by 4% at the udder level for the FAV treatment. The effects were more pronounced in slow milking and low yield quarters resulting in more uniform milking of quarters. Modeled parlor throughput increased by 4% to 7% depending on the percentage of cows that were completely milked in one turn of the parlor. The occurrence of rough teat ends was slightly reduced during the FAV period with no meaningful difference in the occurrence of teats with blue color, palpable rings, or petechia.

A second study was performed using both FAV and Flow Adjusted Stimulation™, (FAS) on a rotary milking parlor. The FAS treatment applied milkline vacuum of 38 kPa, pulsation rate of 50 cycles/min, and pulsation ratio of 30:70 until milk flowrate exceeded 0.5 kg/min at which time a pulsation rate of 60 PPM and ratio of 65:35 was applied and when milk flowrate exceeded 1.6 kg/min milkline vacuum of 48 kPa was applied. The control used the same vacuum settings but did not adjust pulsation settings. Cows that were milked using FAV and FAS had lower odds of short-term teat tissue changes and forced take-off, as well as a higher peak milk flow rate than with FAV alone.

A third study was performed applying FRV at the quarter level in an automatic milking system (DeLaval International VMS). The FRV treatment maintained nominal short milk tube vacuum of 45 kPa throughout milking, while the control was nominally constant vacuum of 45 kPa in receiver, with associated vacuum drop in the milk tube resulting in progressively lower vacuum at the teat-end as milk flowrate increased. The FRV treatment achieved 12% increase in milk harvested per minute of box time, average milk flowrate, 17% increase in peak milk flowrate at the quarter level, and indications of more complete milking. There was no significant difference in post milking teat condition as a result of

several methods employed to manage teat congestion while increasing milking speed:

- Optimized and standardized stimulation and lag times to ensure milk ejection has occurred when teatcups are attached, thus eliminating low flow period at the start of milking.
- Optimized milking intervals in automated milking systems to reduce milking cows with low udder fill.
- Optimized pulsation settings to avoid lost time of extended d-phase duration and increase the milk:rest ratio.
- Development of a liner with effective congestion relief that will support higher milking vacuum levels and milk:rest ratios without producing TEHK or excessive mouthpiece chamber vacuum.
- Timely teatcup removal to eliminate the low flow period at the end of milking, greatly reducing teat tissue stresses that are most pronounced during the low flow / high mouthpiece chamber vacuum period of milking.

The average milk flowrate for high producing US Holstein herds in 1995 was about 2.6 kg/min at the udder level or 0.65 kg/min at the quarter level. The coordinated combination of numerous control technologies described here produced a quarter level average milk flowrate to 1.5 kg/min or approximate doubling over the past 30 years.

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# MILK QUALITY IN RWANDA

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## SUMMARY

Rwanda has made great strides in its dairy industry in recent years due to a committed Government strategy of economic support for the dairy industry, with national budget investment in agriculture increasing from 3.0% in 2006 to 10.1% in 2015 and annual sector growth increasing by 6.0% since 2007 (1).

Annual milk production has increased by more than 98% in the period 2012 – 2021 (2). There has been a similar increase in the National herd increasing by 38% since 2012. Milk production and the size of the national herd have been driven by a number of initiatives between the Government of Rwanda and development partners.

The most recent annual estimate for milk production in Rwanda is from the Rwanda Ministry of Agriculture and Animal Resources (Minagri) 2022 report. This report estimates annual production was 999,976 MT, up 13% in the past 12 months (3).

Inyange commissioned a milk powder plant in 2024, capable of processing around 1,000,000 litres of milk /day.

An increase in annual milk production, combined with the construction of the milk powder plant, producing milk powder for export, has increased the focus on milk hygienic quality in Rwanda.

## INTRODUCTION

Rwanda is one of the smallest and most densely populated countries in Africa. Over 60% of the labour force works in agriculture, with 53.7% of agricultural households keeping cattle.

It is reported that 43% of cattle nationally are of local breeds contributing only 9% of milk production. The Government of Rwanda encourages farmers to increase the proportion of cattle of exotic genetics through genetic improvement drives and the use of artificial insemination, to increase milk production alongside improved animal management and feeding.

Climate related challenges including droughts increase challenges for farmers of sourcing sufficient feed and water. With Rwanda seeing sustained economic growth over a number of years, appetite for dairy related products continues to grow. Further to this, with the opening of a new 1,000,000 l/day milk powder plant in the north-east of the country, the demand for milk nationally is rapidly intensifying.

In the semi-arid, flatter plains of North-Eastern Rwanda, pastoral farmers continue historical and cultural traditions of keeping cattle, whilst grazing these on the grasslands available. These farmers currently lack the knowledge, skills and support services to make the transition from 'cattle keepers' to profitable dairy farmers. 26% of agricultural operators have no formal education and 66% have received only primary level education.

There is a well-established milk production chain with a heavy reliance on farmer owned milk cooperatives. Farmers produce the milk, which they either take to the local Milk Collection Centre (MCC) themselves or they pay a Milk Collector to transport their milk on a bicycle or motorbike to the MCC.

Milk is tested at the MCC and added to the bulk milk cooling tanks at the MCC for onward transportation to the milk processing facility, either in churns or by milk tanker.

Milk collection centres typically are paid 423 RWF / litre (0.22p/l) by the processors and pay the farmers 400 RWF / litre (0.21p/l) therefore operating on very slim margins.

It is estimated that around 50% of milk produced is sold to the MCC and 40% consumed within the household. There is considerable regional variation. It is estimated that a Rwandan cow typically peaks around 10.0 l/ day (4).

## MILK QUALITY CHALLENGES

To more fully understand the milk quality challenges, each section of the milk production chain needs to be examined in more detail, highlighting potential pitfalls and proposing potential solutions.

### Milk production at the farm level

The vast majority of cows in Rwanda are milked by hand. These are either kept in individual pens by small holder farmers who will own a single cow, or by SME farmers who may own a larger herd with up to 20 cows.

Teat preparation is generally limited to a wipe with a damp cloth which is used on multiple cows, followed by fore stripping. Often the milk drawn during fore stripping is used to help clean the teats.

Milk is then harvested into a milk container which is generally plastic and will have been cleaned after the previous milking in cold water with liquid soap. In some cases, hot water is used, which has been heated on an open fire. Water is a scarce resource and the vast majority of farms do not have access to mains (WASSAC) water. Instead, water is either harvested and stored as roof water or collected from the local water course.

Many farms allow the calf to suckle briefly before the cow is hand milked, which makes it very hard to accurately assess the milk production of the cow.
A well-documented source of bacterial contamination of milk is undetected mastitis infections. The Rwanda Youth in Agribusiness Forum (RYAF) is working in partnership with Rwanda Agriculture & Animal Resources Development Board (RAB) carrying out CMT testing on individual farms to identify cows with clinical and sub-clinical infections. Personal communication with the RYAF Project leader suggests that more than 50% of animals tested showed a positive response to the CMT reagent.

Ripple Effect Rwanda (RER) have a long-established farmer training format, where best practise is encouraged within a group of farmers by a trained Project Facilitator (PF), peer farmer and community animal health worker. These individuals organise and facilitate training groups where best practise can be rolled out to a larger group of farmers.

**Potential solutions at a farm level**

There are a number of potential interventions at a farm level.

Ensuring the teats are clean before milking is essential and this can be achieved at the most basic level by ensuring that each cow is cleaned using a single cloth which is thoroughly cleaned in hot water after use. Some farms are being encouraged to pre-dip the teats in an Iodine based teat disinfectant then wipe with the individual cloth.

Strip cups are being provided to allow the farmer to identify abnormal milk. Routine use of the CMT test is also being encouraged with on-going trials to establish whether commonly available washing up detergents can be used as an alternative to a commercial CMT reagent which is considered too expensive.

Milking into a clean milking container requires that the container is cleaned thoroughly after use using hot water and a detergent / disinfectant. A cost-

effective alkaline sanitiser has been identified which can be used in combination with hot water at > $75^0$ C.

Lacking milk cooling facilities, many farms will use the milk from the afternoon milking for domestic consumption and / or selling on the informal market, while milk from the morning milk is taken to the MCC.

A clean milk booklet has been published with clear instructions on best practise at a farm level. This booklet has been translated into Kinyarwanda and supported with simple posters. The PF are introducing these guidelines to the farmer groups.

**Milk Collectors**

The milk collectors travel between multiple farms collecting milk and delivering to the MCC. In many cases, milk from multiple farms is mixed together in a single 50 litre stainless steel container, then transported on the back on a motorbike or bicycle.

Once a collection round is completed, the milk collectors will ride to the MCC milk reception area. Depending on the distances covered, milk can be sat in direct sunlight for a significant period of time with the consequential thermal gain.

In the west of Rwanda, milk is often taken on foot to the MCC, over long distances and rough terrain by the farmer or the milkers (cow boys). Although many of these farmers have been issued with stainless milk containers, they prefer to use plastic containers with tight fitting lids which are more comfortable to carry and don't spill if the container is dropped. However, a plastic container is much harder to clean and ATP testing has highlighted high numbers of bacteria on the internal surfaces.

Once the milk has been accepted at the MCC, some milk collectors will rinse their containers at the MCC using cold water. Although most MCCs have access to mains water, due to the slim operating margins, they are often reluctant to allow milk collectors to clean their containers using mains water, relying instead on stored roof water.

Some milk collectors will wash their milk containers more thoroughly when they return home, with a heavy reliance on liquid soap.

**Potential solutions with Milk Collectors**

A significant hurdle to overcome is cleaning and disinfecting the milk transport containers.

The Milk Collectors handbook, translated into Kinyarwanda, and supported by practical milk collectors training, stresses the need to wash the milk containers using a three-stage cleaning process. Ideally mains water should be used although in the absence of mains water, water should be heated to > $75^0$ C.

Initially, it is suggested the milk container is rinsed, then washed in hot water containing an alkali sanitiser (supplied by the MCC Pharmacy) before a final rinse with mains water.

Thermal gain can be a significant challenge and milk collectors are encouraged to collect milk as early as possible in the morning and keep the time from collection to arrival at the MCC to < 1.0 hr.

As milk volumes increase and MCCs open in the afternoon to receive afternoon milk, this will become a greater challenge and it is likely that farmers will be encouraged to wrap the milk containers in a hessian material which can be kept wet. Evaporation of the water will help cool the milk.

A significant challenge identified is the co-mingling of multiple farms in a single container. A milk collector can have milk from 10 – 12 individual farms in a single container.

The milk collector is encouraged to check the visual appearance and smell of the milk before accepting it from the farmer. However, if the milk looks and smells normal, it is accepted.

Following meetings with milk collectors, it was suggested that any additional testing which could be carried out before the milk is accepted, would help the milk collector. In many cases, the financial penalty associated with milk of poor quality, is borne by the milk collector and not the farmer.

A small pilot project is currently underway where milk collectors have been provided with a test kit containing a lactometer (to measure the milk density – looking to establish whether milk has been skimmed or water added) and an alcohol solution (80% alcohol) to mix with milk and establish rancidity.

**Milk Quality at Milk Collection Centres**

When the MCC is open and receiving milk, depending on the size of the MCC, there can be more than 60 milk containers waiting in milk reception.

Although milk is supposed to go through rigorous testing before it is accepted, when a MCC is busy, some milk may not be subjected to all tests.

When milk first arrives, MCC staff will test each container for smell and visual appearance before using a lactometer to detect whether milk has been adulterated. Each milk container is then alcohol tested to check for rancidity.

Due to the cost of the test, composite samples of a number of milk containers are tested for inhibitory substances rather than individual containers.

If milk is considered satisfactory, it is poured through a fine cloth into a holding vat, where it is pumped to the bulk milk tank.

Once the milk is cooled, it is either then transported to the milk purchaser in stainless milk containers on the MCC lorry or collected by the milk purchaser's tanker.

Bulk milk tanks, milk holding vats and milk transport containers are cleaned after use, using either hot or cold water and liquid soap.

Although all internal milk contact surfaces are required to be cleaned using mains water, some MCCs will try and save money using collected roof water.

Hot water provision can be challenging. Some MCCs have been provided with solar heating systems, although these are generally not able to heat water above $50^0$C and suffer a lack of maintenance. Very few systems are operating 12 months after installation.

Some MCCs will heat water over an open fire although this is potentially dangerous when this water is carried on site. Electric water boilers are a potential solution although running costs and maintenance are cited as objections to this system.

ATP testing of milk holding vats, bulk milk tanks and milk transport containers generally indicate high levels of bacterial contamination indicating poor cleaning practises.

Although all MCCs have refrigerated bulk milk tanks, milk cooling is often inadequate as a result of poor service and maintenance or location of the tank compressors. When milk is not cooled sufficiently, or quickly enough, and is then loaded into milk containers to transport to the milk purchaser, the elevated temperatures lead to accelerated bacterial growth.

**Potential solutions at Milk Collection Centres**

While acknowledging that milk collection centres are busy during milk reception, trying to implement a process could help to ensure that all milk cans are correctly tested.

While the testing carried out checks for presence of inhibitory substances, added water, skimmed milk or rancidity, little testing is undertaken on bacterial contamination.

Most MCCs use Resazurin testing to assess the bacterial levels in the bulk milk tank although the method employed is often flawed leading to incorrect results. Milk is tested using the Resazurin method by the milk purchaser and is the single largest cause of milk rejections in Rwanda.

It is considered essential that milk that fails the Resazurin test is identified earlier in the process and rejected before the entire shipment is rejected.

A 'pen' system has been implemented where milk containers (n = 10) arriving at the MCC are held in a pen. The containers are held in this pen until testing is completed.

A small sample is collected from each container in a pen and this co-mingled milk is tested using Resazurin. MCC staff have been provided with all the equipment and training required to carry out Resazurin testing, including a water bath, test tubes, syringes, distilled water and Resazurin tablets.

If the co-mingled sample passes the Resazurin test, the 'pen' is released and the milk accepted by the MCC. If the sample fails the Resazurin test, the milk is held and each individual can is tested to identify the problem container.

Cleaning and disinfecting milk contact surfaces can be problematic and MCC staff have been trained to clean all surfaces using hot mains water and an alkali sanitiser product. Sodium hypochlorite has been introduced (25ml / 10 l) as a final disinfection.

ATP testing following the improved cleaning regime has shown significant benefits.

MCCs are being encouraged to ensure that all milk cooling equipment is serviced annually and that compressors are located to ensure optimal operation and milk temperatures are recorded regularly during each milk collection session.

When milk containers are transported to the milk purchaser, there is often little shade over the containers. This can lead to a significant increase in milk temperature. This can also be combined with long queues at the milk purchaser.

Providing shade on the lorries and coordinating delivery times to avoid long wats in direct sunshine should all help reduce bacterial replication.

## CONCLUSIONS

While milk quality has historically been a challenge in Rwanda, there is real determination at every point in the milk production process to improve the situation.

Whether this is farmers, milk collectors, MCC Managers or staff there is a collective understanding that milk quality needs to improve and they recognise the important role they play in this journey.

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## ACKNOWLEDGEMENTS

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Project partners in Rwanda include Ripple Effect, World Agroforestry/ICRAF and RAB.

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**NOTES**

**NOTES**

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# HOUSING DESIGN FOR A CHANGING ENVIRONMENT

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## SUMMARY

Bunching is a maladaptive behaviour expressed in dairy cattle when temperatures increase. Bunching and other behavioural changes can be monitored using cow position data derived from cow-worn local positioning sensors. Sensor data allows the tracking of behavioural patterns across large groups and extended time periods. Using sensor data our research group have developed a number of space use metrics including full and core ranges, Inter-cow Distance (ICD) and Nearest Neighbour Distance. Increasing temperature resulted in reductions in range size and ICD. Bunching has also been reported by farmers. With this behaviour they report a range of health, production and welfare issues including elevated respiration rates, increased cell counts, lameness and fertility issues as well as production losses and poor staff morale associated with cow mortality and morbidity.

Barn thermal environments are not homogeneous. Our current study aims to combine the animal space use patterns with data from sensors continually logging temperature, humidity and air quality to identify drivers for behavioural changes associated with thermal stress.

Dynamic thermal modelling has been used to assess the effectiveness of basic mitigations for thermal stress in cattle housing. Mitigations need to be tailored to the area of the farm e.g. passive methods are very effective in the cubicle shed but more energy intensive ventilation methods are required for the milking parlour. Predicted heat wave conditions for 2080 would result in the majority of heat stress cases being at level 4 and the tested mitigations do little to abate this. Future building designs should consider new and innovative methods of mitigating future climate conditions.

## INTRODUCTION

With rising global temperatures and the increasing intensification of dairy production, heat stress is already a major challenge to the welfare of dairy cows and the sustainability of dairy production. Heat stress in dairy cattle causes reduced feed intake and milk yield (1), rumen upsets (2), impaired fertility (3)

and impaired immune function (4), as well as negative affective states including hunger, thirst dehydration, and frustration due to conflicting motivations (5). Dairy cattle modify their behaviour to reduce the negative effects of heat stress including seeking shade (6), increasing water intake (7), and standing to increase effective surface area to reduce thermal loading (8,9). Cows may spatially cluster in hotter temperatures (8) but there have been no detailed studies exploring why this potentially maladaptive behaviour persists indoors.

Prior research into heat stress mitigation in temperate climates has been limited (10), and mitigation methods developed in hotter regions are not always as applicable to the UK due to higher humidity levels (e.g. misting). Heat abatement strategies are typically applied at the barn level without consideration for heterogenous indoor microclimates or individual animal variation (11). With higher humidity and water-use concerns in the UK, designing alternative management and building design adaptations for heat stress mitigation that are practical and cost-effective, but which also account for individual animal variation is critical for a sustainable and resilient UK dairy sector.

The BBSRC funded Heat Stress and Building Microclimates project builds on the research team's previous work on bunching behaviour in dairy cattle (12) and aims to determine the key drivers of spatiotemporal variability in indoor microclimates and how indoor microclimates interact with the behavioural responses of dairy cows. The project also engages with farmers to co-create mitigation strategies that will be tested using thermal building modelling techniques.

## BEHAVIOURAL RESPONSES TO TEMPERATURE VARIATION IN HOUSED DAIRY CATTLE

A real-time local positioning system was used to track the spatial position and activity of a group of approximately 100 high yielding Holstein cows in a commercial dairy cubicle yard on Farm A. Cow position was continuously monitored at high temporal resolution over 4 mo between August and November 2014 (12). On farm B (Centre for Dairy Research, University of Reading), a high yielding group of approximately 110 cows was monitored for 6 mo between June and December 2024. Bunching was determined using 4 different spatial measures determined on an hourly basis: herd full and core range size, mean herd inter-cow distance (ICD), and mean herd nearest-neighbour distance (NND). In addition on Farm B, activity and time in proximity to key barn features such as water troughs and fans were analysed.

For Farm A, when hourly mean ambient temperatures were above 20°C, the herd showed higher bunching behaviour with increasing ambient temperature (i.e., reduced full and core range size, ICD, and NND). Aggregated space-use intensity

was found to positively correlate with localized variations in temperature across the barn (as measured by animal-mounted sensors), but the level of correlation decreased at higher ambient barn temperatures.

Based on preliminary results for Farm B reduced ICD and increased activity were again observed with increasing barn temperature. In addition, time spent in proximity to water troughs and fans was greater with increasing barn temperature.

## MICROCLIMATE VARIABILITY AND POSSIBLE DRIVERS FOR BUNCHING

### Monitoring of building microclimates on case study farms.

The study enrolled six case study farms situated in the southern half of the UK. All farms had reported heat stress related problems and were recruited via members of the project steering group (made up and independent farm advisors, veterinarians, building industry representatives, welfare organisations and policy makers). Each farm was fitted with approximately 20 temperature sensors, 20 temperature humidity sensors, 2 $CO_2$ and 2 $NH_3$ sensors and monitored for a minimum of 3 weeks in the winter and 6 weeks in the summer. Ventilation surveys were conducted on each farm. Preliminary analysis indicated variability in thermal microclimates within buildings and also in barn ventilation. In one building, sensors positioned in direct sunlight recorded temperature of 48°C when those in the rest of the building ranged from 28.4-30.4°C. While this may not be indicative of the cow experience it does suggest that conditions capable of triggering maladaptive behaviours such as bunching are possible.

### Farmer focus group meetings

Participating case study farmers also took part in one of four participatory focus group meetings to which other local farmers were invited. Farmers were asked about their experiences of heat stress. Almost all participating farmers had observed bunching behaviour on their farms. Further to this they reported a range of health and production issues resulting from the maladaptive bunching behaviour including increased cell counts and clinical mastitis, impaired fertility, increased lameness and hampered recovery from other health conditions during high temperatures. Daily and seasonal bunching patterns were described. It was common for bunching behaviour to persist for a significant time after the heat stress conditions had abated. Farmers created diagrams of their farms showing key features which might influence building microclimates. Factors such as air flow, fresh air and direct sunlight were all discussed but when compared across farms there was poor agreement as to the most important driver for bunching behaviour.

## THERMAL MODELLING OF POSSIBLE HEAT STRESS MITIGATIONS

Indoor air temperature and relative humidity for the cubicle yards and milking parlour at the Centre for Dairy Research were recorded every 10 mins between 26th May to 28th July 2021, capturing the 2021 UK heatwave period (16th–23rd July), which marked the fifth warmest July on record (Met Office, 2021). Field measurements of the building dimensions, construction materials and thermal properties were taken and used for a dynamic thermal modelling (EnergyPlus, version 22.1). The model was adapted to account for the internal gains from the dairy cattle using average body weights and yields for the study herd. Table 1 outlines the heat stress classification used. The following heat stress metrics were used to compare the model outputs for the different barn areas and modelled scenarios 1) Heat stress hours, the time above each heat stress threshold, 2) Heat stress risk %, the heat stress hours relative to the total possible hours in that zone (15).

**Table 1 Heat stress classification (13)**

| THI Classification | THI Range | THI level threshold |
|---|---|---|
| **Level 1: Mild Stress** | 68 ≤ THI < 72 | 68 |
| **Level 2: Moderate Stress** | 72 ≤ THI < 80 | 72 |
| **Level 3: Severe Stress** | 80 ≤ THI < 90 | 80 |
| **Level 4: Emergency** | 90 ≤ THI | 80 |

Future heatwave predictions were made using Example Extreme Weeks developed by Coley et al. (14).

The effectiveness of various mitigation measures at reducing the heat stress risk and heat stress duration were tested against the 2021 heat wave data and the predicted 2080 heat stress event. In 2021, total heat stress hours i.e. THI $\geq$ 68 were 139 for the cubicle yards and 55 for the parlour. With heat stress risk 72% for the cubicle yards and 86% for the parlour with no animals in level 4 (emergency). A passive mitigation of reducing solar gain by painting the roof white was most effective in the cubicle shed where roof area was large. However, employing all available ventilation in the parlour was most effective. Predicted

heat wave conditions in 2080 would result in 100% risk of level 4 heat stress in the milking parlour with no mitigations. Even the most successful mitigations would only reduce the risk to 40% level 3 and 60% level 4 (15).

## CONCLUSIONS

The project results to date emphasize the urgent need to develop effective and sustainable mitigation strategies against future climatic conditions. These will almost certainly require some novel and innovative building designs for dairy and other livestock. These designs should take into account the needs of the cow in terms of health, welfare, and the need to express behaviours, as well as needs of farm staff.

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**NOTES**

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# MASTITIS CONTROL PLAN CASE STUDY

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## SUMMARY

Arnside Tower Farm is a medium sized spring calving herd based in Lancashire. Clinical mastitis was well controlled, but subclinical mastitis was a concern, with high bulk cell count in early lactation. This paper describes a Mastitis Control Plan, with initial data assessment, action plan and outcomes. As a result of the Plan, cell count is much better controlled, and staff morale is improved.

## INTRODUCTION

Arnside Tower Farm milks 250 crossbred cows in a spring calving system. They milk twice per day, average 5700 litres with 450kg milk solids. 90% calve in the first 6 weeks of the season (February and March). Cows are paddock grazed day and night from calving to dry off, they are all dried off on the 1st of December and sent to 2 other units. The farm presented in August 2023 with a high bulk milk SCC.

Aims of the Mastitis Control Plan:

- Investigate dry cow management – some question marks over one of the contract farmers who is housing dry cows over winter
- Investigate high cell counts in the spring
- Understand parlour routine – new staff member starting soon and parlour alterations happening in December

## MATERIALS & METHODS

Data was taken from the milk recording organisation (Quality Milk Management Services) and analysed in TotalVet©. Following data analysis, a full AHDB Mastitis Control Plan was carried out.

## RESULTS

### Diagnosis

Figure 1 shows the Mastitis Pattern Analysis Report. The pattern was 'Mixed Environmental' with the dry period and lactating period being more important at

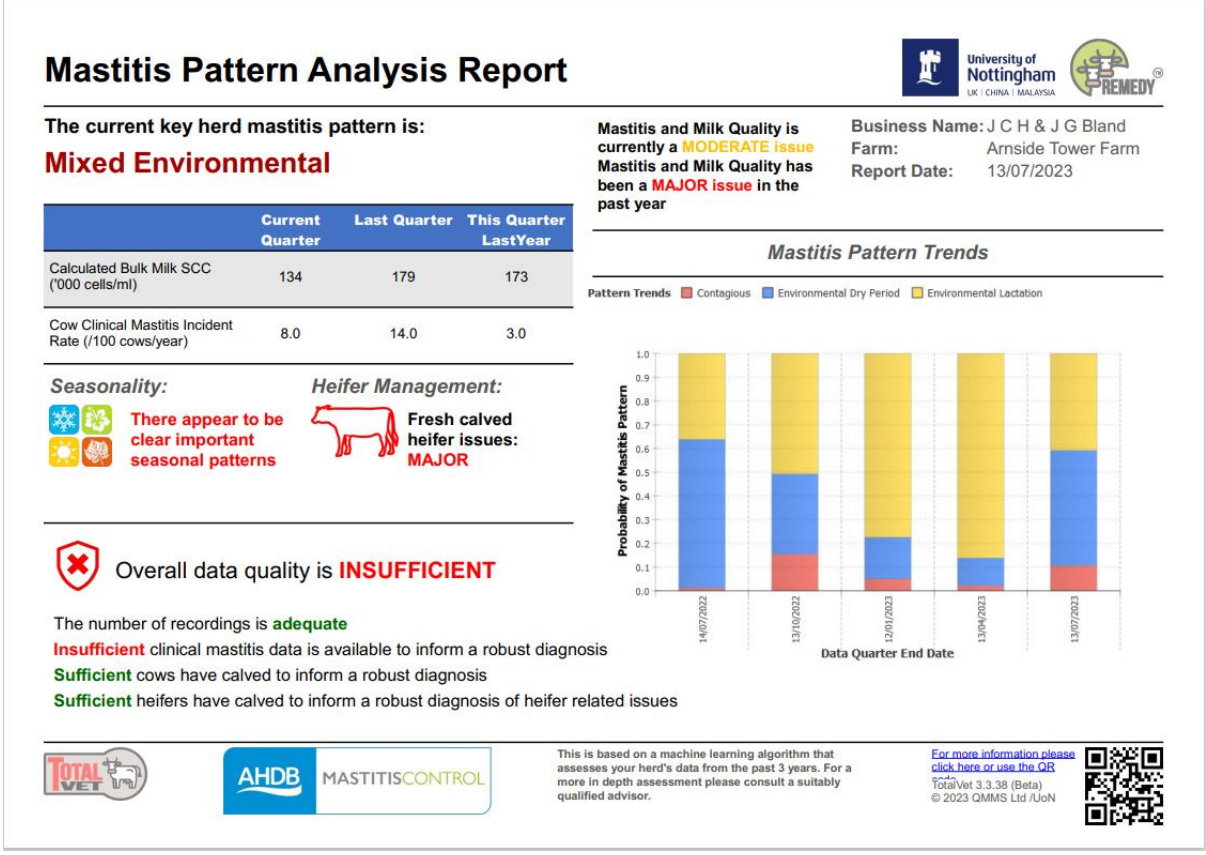
different times of the year. Clear seasonal patterns were identified and problems with heifer mastitis were also present.

The rate of clinical mastitis was low (10 cases/100 cows/year) – this either reflects under-detection or excellent control of new infections. Bulk somatic cell count averaged 160, but could be >300,000 cells/ml in the spring.

New high cell count infections were above target both during the dry period and lactation. In the 2023 calving season, 25% of cows had a high SCC at the first milk recording of lactation (Fresh Calver Infection Rate, Target <10%). Approximately 6-7% of cows developed new high cell counts during lactation (Lactation New Infection Rate, target <5%).

Given the time of year (August 23) it was decided to focus on Environmental Lactation infections, and revisit dry cow management ahead of drying off in December.

**Figure 1 Mastitis Pattern Analysis Report from August 2023**



**Actions**

The MCP identified some key areas to focus on in order to manage Environmental Infections during lactation.

- Review parlour routine (inconsistent foremilking, inadequate teat spray coverage, 1 towel per cow, reduce lag time to 90 secs)
- Adjust strings on the ACR so that clusters sit better and don't hit the ground
- Rubber mats in the parlour and on exit to promote milking efficiency
- NSAIDs for all cases of clinical mastitis
- Narrowed standings in the parlour to improve cow cleanliness

Actions focussed on Environmental Dry Period infections (to revisit in late 2023/early 2024)

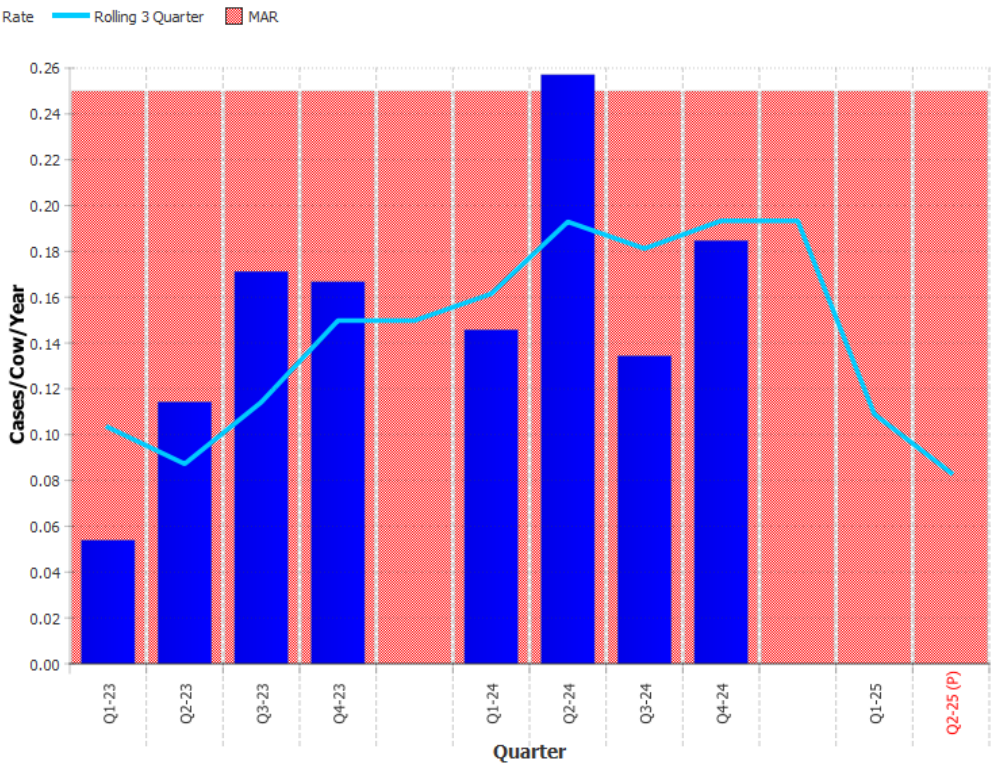
- CMT all fresh cows at the first milking
- Change contract farm so that dry cow management is consistent and only at one site

**DISCUSSION**

Of the 7 points in the action plan, 6 have been implemented by Spring 2025. The only point that has not yet been addressed is to CMT all fresh cows at the first milking.

Figure 2 shows the quarterly rate of clinical mastitis (cases/cow/year). The rate of clinical mastitis increased during 2024, from 0.1 cow cases to 0.2. It should be highlighted that the new herdsperson is carrying out foremilking at every milking, and so the apparent increase more likely reflects better detection rather than worsening udder health. In the same time, the bulk SCC remained low, and has averaged 141,000 cells/ml in the past 12 months.

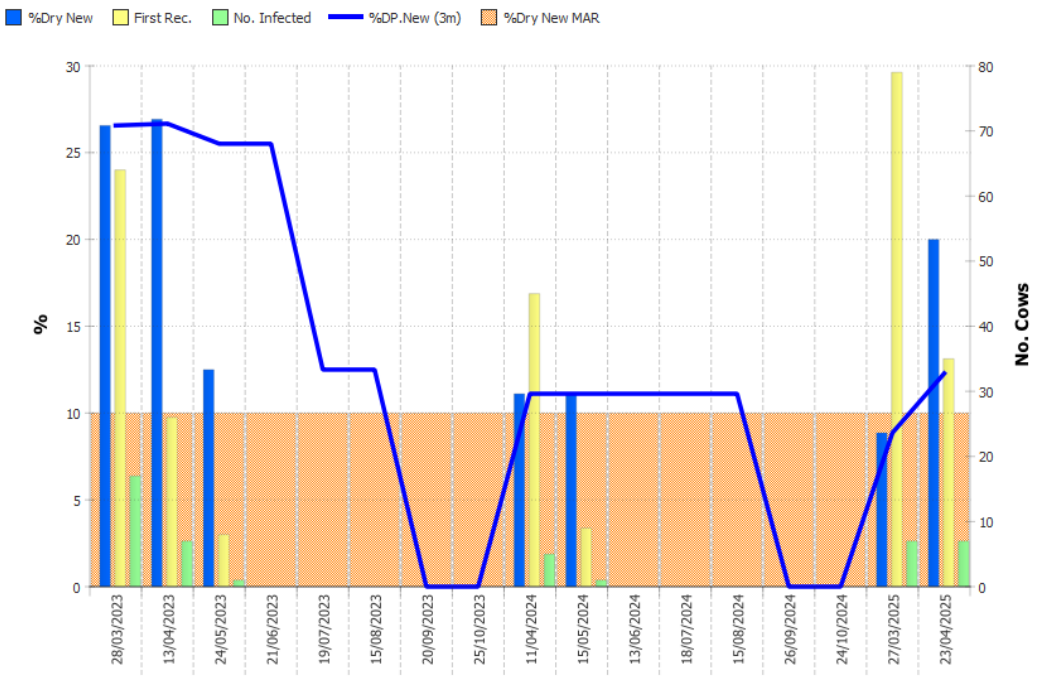
**Figure 2 The quarterly rate of clinical mastitis (cases per cow per year). Case rate in each quarter is shown by the blue bars, with a rolling 3 quarter average rate plotted as a line. The case rate is shown relative to a target of <0.25 cases per cow per year**




The key improvements came in subclinical mastitis:

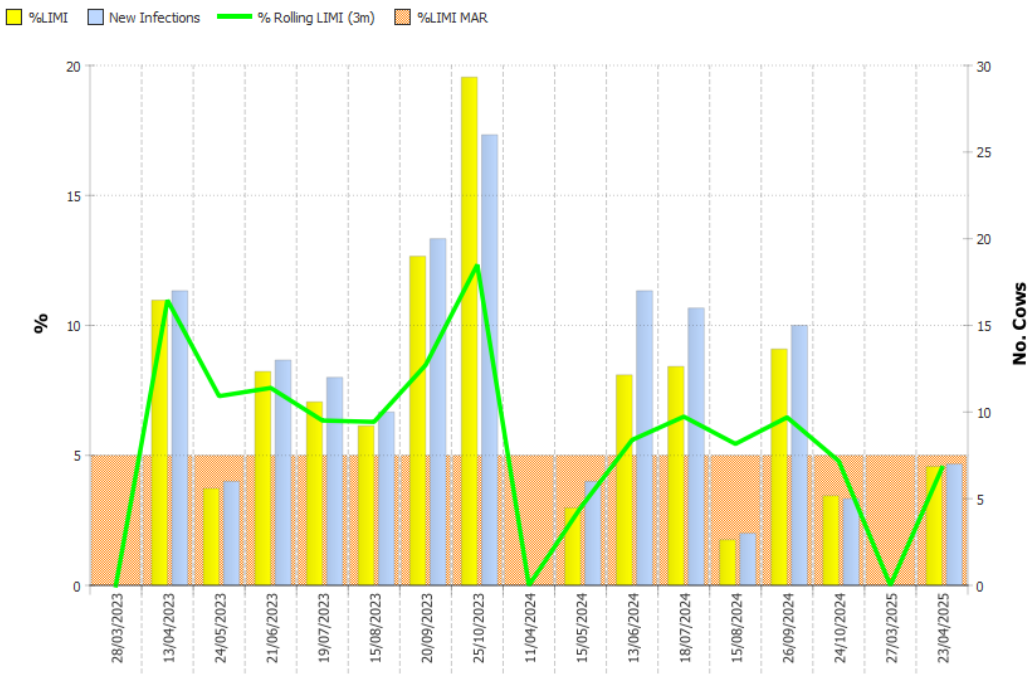
- In spring 2024 and 2025, dry period infections were much better controlled. Figure 3 shows the Dry Period New Infection Rate (DPNIR), which dropped from 26% in 2023 to 11% in 2024 and has stayed low (12%) in early 2025 (Figure 3).

**Figure 3 The Dry Period New Infection Rate. Bars show the number of cows eligible for a new infection each month (yellow), and the number infected (green). The blue bars show the DPNIR in each month, and the blue line shows the rolling 3-month average, which can be interpreted as the DPNIR for that season**




- Figure 4 shows the Lactation New Infection Rate (LNIR) also dropped during the same period, from 8-9% in 2023 to 6% in 2024.

**Figure 4 The Lactation New Infection Rate. Bars show the number (grey) and proportion (yellow) of cows developing a new infection >30 days in milk at each milk recording i.e. moving from a low to a high SCC during lactation. The green line shows the rolling average lactation new infection rate.**




## CONCLUSIONS

Following implementation of the Mastitis Control Plan, udder health is under better control. This has largely been achieved by getting all members of the farm team to work together. As a result of better udder health, the farm moved to 10:7 milking in September 2024 (i.e. milking 10 times in 7 days), and once daily milking in October 2024, with no negative impact on milk solids or cell count. This has given the team more time to carry out other jobs on farm, and greater job satisfaction. Going forward, the farm is now more confident to move to 10:7 milking earlier in the season, though this depends on milk production.

The 2025 calving season has got off to a good start, with only 3 cases of clinical mastitis so far. It is important to continue to monitor udder health over the coming months.

The farm is planning to put in an automatic wash system in the parlour. This is unlikely to have a direct impact on udder health, but will speed up milking. It may also reduce Bactoscan results. The farm is also planning to make changes to the parlour exit, to improve cow flow and milking efficiency.

**NOTES**

**NOTES**

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*Proceedings of the British Mastitis Conference (2025) Sixways, Worcester, p 61 - 63*
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# KEY PERFORMANCE INDICATORS OF UDDER HEALTH IN 320 UK HERDS RECEIVING AUTOMATED MASTITIS PATTERN REPORTS IN 2024

**K.A. Leach[1], A. Manning[1], K. Bond[2], J. Mathie[3], and A.J. Bradley[1,4]**
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In 2021, an automated Mastitis Pattern Analysis Tool (MPAT) was developed, through the REMEDY project, supported by InnovateUK. Farmers milk recording with QMMS, NMR or CIS can register to receive an MPAT report each time they milk record, which gives some key performance indicators (KPIs) and highlights the predominant mastitis pattern on farm. This provides an easy monitoring system and indicates where control efforts should be directed. Since 2022, over 400 farms have signed up, creating an anonymised population of herds in which udder health can be monitored both retrospectively and currently. This poster summarises KPIs for udder health for the calendar years 2021 and 2024, in 320 herds that received a Mastitis Pattern Report during November or December 2024.

Inclusion criteria were at least four milk recordings in both 2021 and 2024, with the latest being within eight weeks of the end of the year, and, for analysis of clinical mastitis, a reported rate of at least 5 cases per 100 cow per year, in 2021 and 2024. Udder health was assessed using indicators of subclinical mastitis, based on individual cow somatic cell count (ICSCC), and clinical mastitis (where record quality allowed). Parameters in the form of 12 month averages were calculated in TotalVet©. The change between 2021 and 2024 was analysed using the Wilcoxon signed rank test.

There were small but statistically significant improvements in the medians ($p < 0.05$) for the proportion of chronically infected cows (8.5% to 7.9%) and cows over 200,000 cells/ml (14.9% to 14.3%), lactation new infection rate (6.7% to 6.1%) and dry period new infection rate (14.2% to 13.0%) and also in the rate of clinical mastitis originating from the dry period (0.50 to 0.43 cows in 12). However, the overall rate of clinical mastitis altered little, from 22 to 21 cases per 100 cows per year. The KPIs recorded for 2021 agree well with those reported for a previous group of "Sentinel Herds" that year (e.g. medians of 14.3% for proportion of cows over 200,000 cells/ml, 14.1% for dry period new infection rate, 0.49 cows in 12 infected in the dry period, and 21 clinical cases per 100 cows per year [1]. This suggests that the "MPAT" herds represent a similar subset of the national herd.

The proportion of herds reporting clinical mastitis cases at a rate greater than 5 cases/100 cows per year increased from 49% in 2021 to 68% in 2024 and only 19 herds that had been reporting plausible clinical rates in 2021 ceased to do so. It is possible that use of the MPAT has stimulated understanding of the value of recording clinical cases and monitoring clinical and subclinical mastitis.

**Table 1 Key farm indices and udder health indicators 2024 and comparison with 2021**

Significance of difference between 2024 and 2021 * p <0.05, ** p<0.01, *** p <0.001 (Wilcoxon signed rank test)

| Variable | n | Mean 2024 | 1st Q 2024 | Median 2024 | 3rd Q 2024 | Median 2021 |
|---|---|---|---|---|---|---|
| **Herd size** | 320 | 264 | 145 | 215 | 305 | 206 |
| **Mean annual rolling 305 day yield (l)** | 320 | 8522 | 7205 | 8537 | 9712 | 8548 |
| **Calculated bulk milk SCC (,000/ml)** | 320 | 168 | 133 | ** 161 | 197 | 168 |
| **Clinical mastitis (CM) rate (cows affected /100 cows/ year)** | 138 | 23.7 | 13.8 | 20.7 | 31.2 | 22.5 |
| **Dry period origin CM rate (cows in 12)** | 138 | 0.48 | 0.29 | 0.43 | 0.61 | 0.50 |
| **Lactation origin CM rate (cows in 12)** | 138 | 1.7 | 1.1 | 1.6 | 2.2 | 1.7 |
| **Lactation new infection rate (%)** | 320 | 6.6 | 4.6 | *** 6.1 | 8.1 | 6.7 |
| **Dry period new infection rate (%)** | 320 | 13.9 | 9.4 | * 13.0 | 17.1 | 14.2 |
| **Dry period cure rate (%)** | 320 | 80 | 74 | 81 | 87 | 80 |

| Variable | n | Mean 2024 | 1st Q 2024 | Median 2024 | 3rd Q 2024 | Median 2021 |
|---|---|---|---|---|---|---|
| **Fresh calver infection rate (%)** | 320 | 15.0 | 10.4 | * 14.1 | 18.4 | 15.3 |
| **% chronically infected** | 320 | 8.6 | 5.9 | * 7.9 | 10.7 | 8.5 |
| **% > 200,000 cells/ml** | 320 | 14.8 | 11.0 | ** 14.3 | 18.0 | 14.9 |

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# IMPORTANCE OF HEIFERS IN MASTITIS CONTROL

**A. Manning[1], K.A. Leach[1], K. Bond[2], J. Mathie[3], and A.J. Bradley[1,4]**
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Mastitis control is improving across UK farms, as shown by reducing rates of clinical mastitis and bulk SCC [2,4], though no UK studies have specifically evaluated heifer mastitis parameters. Heifers generally have lower somatic cell count (SCC), and lower rates of clinical mastitis, but new infections during the first lactation remain important as they may affect future performance and profitability [1]. The aim of this study was to summarise udder health performance in heifers across UK farms.

The REMEDY platform collates data from more than 1,000 UK dairy farms, of which 400 have opted to receive the Mastitis Pattern Analysis Reports [3]. Data from all farms going through the REMEDY platform were anonymised and benchmarked on the 31st of December 2024. Farms were excluded from clinical mastitis calculations if they had an incidence of fewer than 5 cases per 100 cows per year. Farms were excluded from subclinical mastitis calculations if they had fewer than 4 milk recordings in 2024, or if they hadn't had a milk recording within 2 months of the end of 2024. Key mastitis parameters in heifers were calculated using TotalVet© – 12-month averages were used to remove any seasonality.

- Clinical Mastitis Rate – incidence of clinical mastitis /100 cows / year
- Dry Period Origin clinical mastitis (DPO) – incidence rate of index cases occurring within the first 30 days of lactation
- Lactating Period Origin clinical mastitis (LPO) – incidence rate of index cases occurring between 31 and 305 days of lactation
- Prevalence of infection (>200K) – proportion of heifers with a somatic cell count >200,000 cells/ml at each milk recording
- Prevalence of chronic infections (% chronic) – proportion of heifers that are persistently high cell count at each milk recording
- Fresh Calver Infection Rate (FCIR) – proportion of heifers with a high somatic cell count (>200,000 cells/ml) at their first milk recording ≤30 days in milk
- Lactation New Infection Rate (LNIR) – rate at which uninfected heifers at the previous milk recording cross the 200,000 cells/ml threshold i.e. become infected >30 days in milk.

Table 1 shows the proportion of herds above target for key udder health parameters. Rates of clinical mastitis were low (on the farms with records), 95.8% of farms had a rate of heifer mastitis below target. When mastitis incidence was broken down by the period in which it occurred, DPO was above target on 10.6% of farms, LO was above target on 2.6% of farms.

On most farms, the prevalence of subclinical mastitis was low, with few farms having a high proportion of heifers with chronic high cell counts. Despite this, the fresh calver infection rate was above target in the majority of farms. New infections during lactation were better controlled, but were above target in around a third of farms.

**Table 1. Key udder health parameters for first lactation heifers on UK farms**

| Clinical mastitis (n=189) | | Minor problem | Moderate problem | Major problem |
|---|---|---|---|---|
| Clinical Mastitis Rate per 100 cows per year | *Target* | <25 | 25-50 | ≥50 |
| | | 181 (95.8%) | 8 (4.2%) | 0 |
| DPO | *Target* | <1 cow in 12 | 1-2 cows in 12 | ≥2 cows in 12 |
| | | 169 (89.4%) | 16 (8.5%) | 4 (2.1%) |
| LO | *Target* | <2 cows in 12 | 2-4 cows in 12 | ≥4 cows in 12 |
| | | 184 (97.4%) | 5 (2.6%) | 0 |
| Subclinical mastitis (n=370) | | Minor problem | Moderate problem | Major problem |
| >200k | *Target* | <20% | 10-20% | ≥20% |
| | | 261 (70.5%) | 104 (28.1%) | 5 (1.4%) |
| >% chronic | *Target* | <5% | 5-10% | ≥10% |
| | | 272 | 85 | 13 |
| FCIR n = 370 | *Target* | <10% | 10-20% | ≥20% |
| | | 123 (33.2%) | 173 (46.8%) | 74 (20%) |
| LNIR n = 370 | *Target* | <5% | 5-10% | ≥10% |
| | | 252 (68.1%) | 109 (29.5%) | 9 (2.4%) |

These results show that clinical mastitis in heifers is better controlled than subclinical mastitis. Rates of new infection appear to be higher in fresh calvers, compared with the rest of lactation as shown by high DPO and FCIR. High rates of infection in early lactation don't appear to drive high prevalence of chronics, but could have a negative impact on milk production, performance and longevity [1].

This research demonstrates that clinical mastitis in heifers is well controlled on the majority of UK dairies. Subclinical mastitis is less well controlled, particularly new infections in the first 30 days in milk. Herds with high rates of infection in heifers are likely to benefit from targeted advice, particularly focussed on the pre-calving environment.

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# EFFECTIVE PRE-MILKING HYGIENE PROTOCOLS WILL CONTRIBUTE TO REDUCE CONTAMINATION OF THE MILKING EQUIPMENT, MILK AND COWS

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## SUMMARY

The objective of this research was to investigate and illustrate the effects of pre-foaming with lactic acid (Kenopure Pro™) on the teat skin colonisation in dairy cows, utilizing both *in vitro* and *in vivo* methods. In an *in vitro* study, 16 rubber calf nipples were assigned to 8 treatments in duplicate. Four rubber calf nipples served as negative controls and were not exposed to *Streptococcus uberis*. The four other calf nipples were exposed to *Streptococcus uberis* and not prepared, only wiped with a dry and clean paper towel, pre-foamed with Kenopure Pro™ and immediately wiped with a dry and clean paper towel, or pre-foamed with Kenopure Pro™ and wiped with a clean and dry paper towel after a contact time of 30 seconds. In an *in vivo* setting, 64 teats of 16 cows were assigned to the same four preparation methods as in the *in vitro* trial. The *in vitro* showed that pre-foaming with lactic acid was more effective in reducing *Strep*. spp. from the teat skin than wiping with a dry paper towel alone. Additionally, the *in vivo* study confirmed that wiping the teats with a dry paper towel alone is insufficient to remove *Streptococcus* spp. and *Staphylococcus* spp. from the teat skin of (highly) contaminated teats.

## INTRODUCTION

Teat cleaning and disinfection helps remove visible dirt, manure, and bacteria from the teat skin, significantly reducing the risk that bacteria will become dislodged during milking and enter the teat canal, potentially causing new udder infections (2).

## MATERIALS & METHODS

In the *in vitro* study, 16 rubber calf nipples were assigned to 8 treatments in duplicate (Table 1). Four rubber calf nipples were soaked in 80 ml of sterile brain heart infusion medium and served as negative controls. The other four rubber calf nipples were soaked in in a bacterial solution of *Streptococcus uberis* ATCC19436 [1.75 x $10^9$ colony forming units (cfu)/mL]. After 15 minutes, teats were air dried for 10 minutes and prepared as described in the Table 1. One duplicate was left on a paper towel to swab the outside of the teat. The swab was

plated on Esculin Blood agar. The other duplicate was immersed for 30 minutes in 80 ml sterile PBS to soak off residual bacteria. Of this solution, 1 ml was then plated on Esculin Blood agar and a serial dilution was made to perform colony count (1 ml is plated). In the *in vivo* study, 16 cows were included. Per cow, one teat served as control which was not cleaned before swabbing (i.e. 2 sec. on each side of the teat). The three other teats were treated as presented in Figure 1.

**RESULTS**

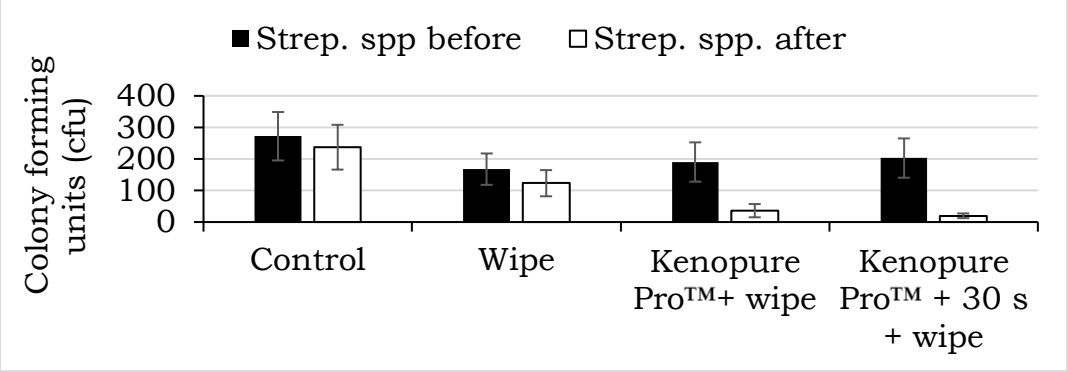
The main findings of the *in vitro* study are summarized in Table 1. All swabs of all negative control rubber calf nipples remained culture-negative, independent of the teat preparation method.

**Table 1 *In vitro* comparison between different teat preparation methods**

| Teat | Exposure | Preparation | Outside[4] | Dilution[5] |
|---|---|---|---|---|
| 5 | Yes[1] | No cleaning | Positive | 1.2 x $10^4$ cfu/ml |
| 6 | Yes | Dry paper towel | Positive | 70 cfu/ml |
| 7 | Yes | Pre-foaming + immediate[2] | Negative | 0 cfu/ml |
| 8 | Yes | Pre-foaming + 30-seconds[3] | Negative | 0 cfu/ml |

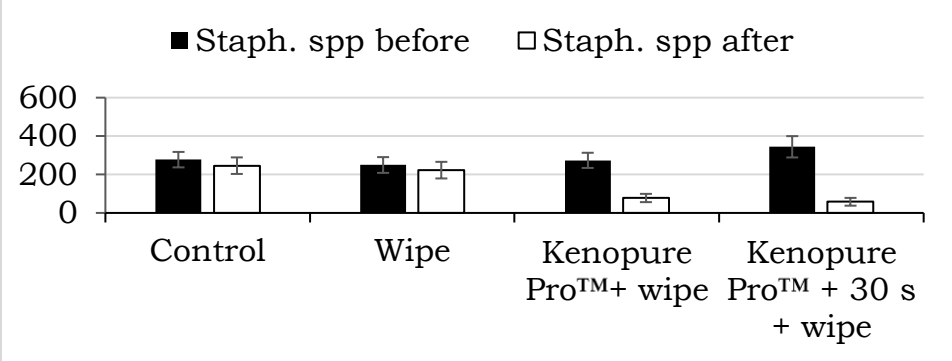
[1]Soaked in a bacterial solution of *Strep. uberis* ATCC19436 (1.4 x $10^9$ cfu/ml). [2]Immediately wiped with a dry towel after pre-foaming with Kenopure Pro™. [3]Wiped with a dry towel after 30 seconds contact time with Kenopure Pro™. [4]Swab of outside of rubber calf nipple. [5]Serial dilution of 1 mL of immersion solution in which rubber calf nipple was soaked for 30 min.

**Figure 1. Average colony forming units of *Streptococcus* spp. (+/- standard error of the mean) before and after teat preparation in the different teat preparation groups.**



The *in vivo* study demonstrated a high bacterial load on the teat skin before teat preparation with strong variation among the teats. The average (min-max) for *Strep*. spp. was 272 cfu (34-866) for control teats, 168 cfu (0-876) for teats that were wiped only, 190 cfu (19-836) for teats that were pre-foamed and immediately thereafter wiped, and 203 cfu (14-814) for teats that were pre-foamed and wiped after a contact time of 30 seconds. For *Staph*. spp., the average (min-max) cfu for the different preparation methods was 277 cfu (111-657), 249 cfu (18-692), 274 cfu (78-623), and 344 cfu (94-924), respectively. Also, pre-foaming with Kenopure Pro™ appeared to be more effective in reducing the high bacterial load of *Strep*. spp. and *Staph*. spp. on the teat skin compared to wiping alone, especially when a contact time of 30 seconds was maintained as shown in Figure 1 and 2.

**Figure 2. Average colony forming units of *Staphylococcus* spp. (+/- standard error of the mean) before and after teat preparation in the different teat preparation groups.**




## DISCUSSION & CONCLUSION

In the *in vitro* study, *Strep. uberis* bacteria adhered to rubber calf nipples comparably to cow teats. Wiping with a dry paper towel alone reduced the bacteria count, but pre-foaming with Kenopure Pro™ proved more effective, regardless of the contact time. Consistent with other findings (1), teat skin colonisation by both *Staph*. spp. and *Strep*. spp. decreased more after pre-foaming than after wiping with a paper towel alone. It can be concluded that wiping the teats with a dry paper towel alone is insufficient to remove *Strep*. spp. and *Staph*. spp from the teat skin of (highly) contaminated teats.

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# *E. COLI*, *STAPH AUREUS*, *E. FAECALIS*, *P. AERUGINOSA* AND *L. PNEUMOPHIL:* KILL EFFICACY OF OXI-TECH's PULSE OXIDATION CELL SYSTEM

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## SUMMARY

Oxi-Tech Solutions' *Pulse Oxidation Cell* system is a very effective and efficient way of killing waterborne bacterium. For most bacteria, 4 log reductions were witnessed using the lowest ozone level (0.5ppm) and the minimum contact time (30 seconds) tested. This combined with the environmental advantages of reduced biocide usage, no residual output to wastewater or soil and potential reductions in carbon footprint, demonstrate the benefits of the system over current traditional methods.

## INTRODUCTION

A series of tests were carried out to determine the log reduction of selected range of bacteria found in clean in place disinfection systems using Oxi-Tech's *Pulse Oxidation Cell* system which creates variable ozone concentrations in water to be used for cleaning and disinfecting purposes.

## MATERIALS AND METHOD

The tests were conducted at three different ozone levels (0.5, 0.8 and 1.0ppm) and three different contact times (30 seconds, 2 minutes and 5 minutes). The bacteria used for the experiment were those of interest or concern for clean in place disinfection systems: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Legionella pneumophila*. The target value set for the log reduction of bacteria was 4 log or a 99.99% reduction.

## RESULTS AND CONCLUSION

Results showed that the *Pulse Oxidation Cell* system can reach the target using 0.5ppm of ozone with a contact time 30 seconds for most bacteria tested in this experiment. With increased ozone levels and contact times the log reduction of bacteria increased with 5 log reductions also being recorded.

An ozone concentration of at least 0.8ppm for a minimum five minutes should be recommended to confidently achieve a 4 log reduction across all types of bacteria as used in this experiment.

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# THE MILK MICROBIOTA AND ITS ASSOCIATION WITH MASTITIS IN DAIRY CATTLE

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## SUMMARY

Mastitis is a common disease in dairy cattle with significant economic and welfare implications. The associations between milk microbiota — the community of bacteria, fungi, viruses, and archaea in milk — and mastitis are not yet fully understood. This study examined the link between the milk microbiome and mastitis by analysing milk from healthy cows (H, n=10), cows with subclinical mastitis (S, n=10), clinical mastitis (C, n=12), and repeated clinical mastitis (R, n=10) in Holstein Friesian cattle. Mastitis causative pathogens were identified via culturing. Microbiome profiles were characterised based on next-generation shotgun sequencing. Microbial diversity (alpha diversity) did not differ significantly across groups. *Clostridium* was the most abundant genus overall but was less dominant in healthy samples. In clinical cases with fungal pathogen culture (n=3), microbial evenness was significantly higher. Notably, repeated clinical mastitis cases showed lower levels of known mastitis pathogens (*Streptococcus*, *Escherichia*, *Raoultella*) compared to single clinical cases, and the largest number of microbial differences were observed between these groups. Genera such as *Limosilactobacillus*, *Dietzia*, and *Propioniciclava* were more abundant in healthy and subclinical cases than in clinical or repeated cases, indicating that these microbes may have a protective role preventing disease progression.

## INTRODUCTION

Mastitis is a prevalent disease of dairy cattle that causes economic losses through decreased milk yield, discarded milk during treatment, and increased veterinary costs. Animal welfare is also negatively impacted due to painful clinical signs such as inflamed and swollen udders. The milk microbiome, the microbiota (bacteria, fungi, viruses, archaea) in milk and their interactions within the milk environment, is altered during mastitis. However, the extent of this impact has not yet been fully explored. The aim of this study was to examine the milk microbiota of animals with different mastitis health status.

## MATERIALS & METHODS

This study was conducted at the SRUC Dairy Research and Innovation Centre, UK, using animals from the Langhill herd. We investigated the association between the milk microbiota and mastitis health status by analysing milk samples from adult, lactating Holstein Friesian animals. Animals were classified as healthy (H, n = 10), subclinical (S, n = 10), clinical (C, n = 12), or repeated clinical (R, n = 10) cases. Health status was determined over a single lactation. Healthy animals had no recorded mastitis cases and at least two consecutive somatic cell counts (SCC) <100,000 cells/ml. Clinical animals were diagnosed by farm staff from visual signs and sampled during their first mastitis episode of the lactation prior to treatment administration. Repeated clinical animals were diagnosed by farm staff again and sampled during a mastitis episode that was not the first of the lactation. Subclinical animals showed no clinical signs of mastitis but were identified through elevated SCC (> 200,000 cells/ml) during routine fortnightly milk recording. All samples underwent mastitis diagnostic culture at SRUC Veterinary Services and microbial DNA shotgun metagenomic sequencing on an Illumina NovaSeq at Edinburgh Genetics.

## RESULTS AND DISCUSSION

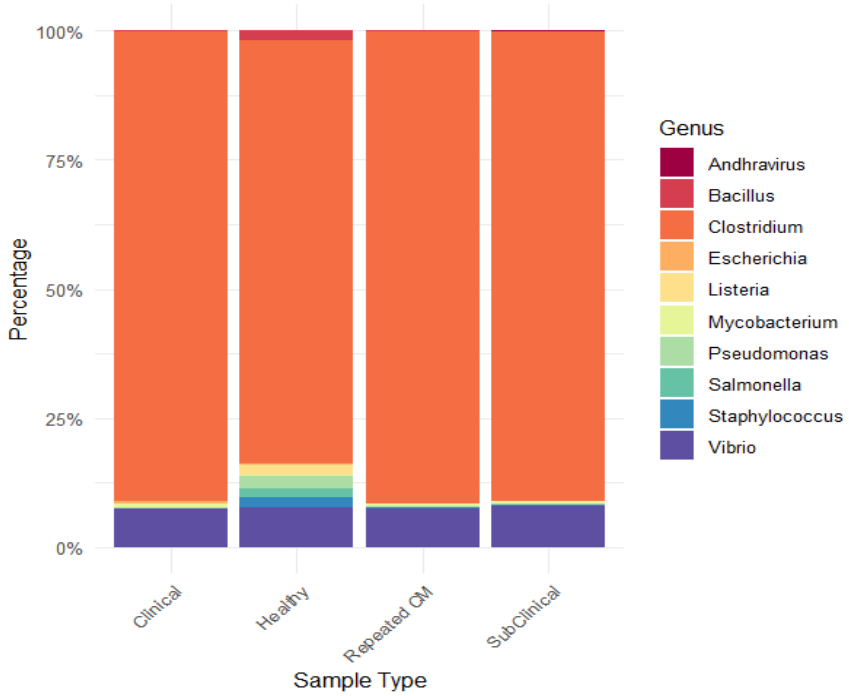


**Figure 1 Relative abundance of the top 10 most abundant genera from Clinical (n= 12), Healthy (n=10), Repeated Clinical (n=10) and Subclinical (n=10) samples. Metagenomic shotgun sequencing data was grouped based on mastitis health status determined from visual observation and somatic cell count.**

Microbial alpha diversity did not differ significantly between sample types. Amongst the top 10 most abundant genera, *Clostridium* was most dominant in all sample types but less abundant in H (fig. 1). In C cases where a fungal pathogen was cultured (n = 3), there was significantly greater evenness (adjusted Shannon diversity $p < 0.001$, Simpson diversity $p = 0.02$). Bray-Curtis dissimilarities were significantly different between mastitis sample types ($p = 0.042$), however, post-hoc pairwise comparisons were non-significant ($p > 0.05$). Milk from R samples contained lower abundances of known mastitis causing pathogens, including *Streptococcus*, *Escherichia*, and *Raoultella*, than C samples. The number of taxa significantly differentiating C and R was noticeably higher than in other pairwise comparisons, with most of these taxa being overrepresented in C (71 taxa) compared to R (2 taxa). This observation is potentially due to antibiotic administration received in the R group for a previous mastitis incident.

*Limosilactobacillus*, *Dietzia*, and *Propioniciclava* were found to be more abundant in H than in C or R samples. They were also found to be more abundant in S in clinical samples, suggesting a negative association with disease progression.

## CONCLUSIONS

The milk microbiota of animals with differing mastitis status were significantly different. Whilst overall milk microbiota was dominated by *Clostridium*, samples where fungi were identified as the causative pathogen were significantly less dominated by this genus. *Limosilactobacillus*, *Dietzia*, and *Propioniciclava* were overrepresented in healthy and subclinical mastitis cases compared to clinical and repeated clinical mastitis, a negative association with disease progression. Future work will explore the functional potential of the milk microbiome in these samples and further investigate microbial features that differ across the mastitis health status groups.

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**NOTES**