IDF MASTITIS AND MILKING TECHNOLOGY JOINT CONFERENCE

11-13 MARCH, 2026 | STOCKHOLM

PROCEEDINGS

Swedish University of Agricultural Sciences, SLU
Department of Applied Animal Science and Welfare
Reports from department of applied animal science and welfare, no. 15
2026

IDF Mastitis and Milking Technology Joint Conference 2026 Proceedings

10 – 13 March 2026

Edited by

Dr. Hasitha Priyashantha, Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, 81100, Sri Lanka

Dr. John Upton, Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 C997, Ireland

Prof. Sigrid Agenäs, Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, SLU. Po Box 7024, 750 07 Uppsala, Sweden

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Foreword

Mastitis remains one of the most persistent and complex challenges facing the global dairy sector. As a disease that directly affects animal health and welfare, milk quality, and farm productivity, its relevance extends well beyond the farm gate. Addressing mastitis is therefore fundamental to safeguarding the production of safe, nutritious dairy foods and to sustaining the livelihoods of the more than one billion people who depend on the dairy sector worldwide. Central to this challenge is the close interconnection between udder health and milking technologies. Health, welfare, and productivity are intrinsically linked to how cows are milked, monitored, and managed.

Over recent decades, advances in milking technologies have not only enhanced efficiency and productivity, but have also contributed to improved animal health outcomes. In parallel, progress in mastitis prevention, detection, and treatment has created new opportunities to reduce disease incidence and severity, supporting healthier cows and more resilient, sustainable dairy systems. Such progress is neither accidental nor static. It is driven by science, innovation, and collaboration across disciplines, regions, and sectors. The dairy sector must continuously evolve, informed by robust scientific evidence and supported by the exchange of knowledge and experience. In this context, an ever-developing science base is essential to respond to emerging challenges, technological advances, and societal expectations.

The International Dairy Federation plays a central role in supporting this collective endeavour. Guided by its vision to nourish the world by advancing dairy knowledge, IDF works as a trusted global platform where science underpins dialogue, consensus-building, and action. None of this would be possible without the dedication and expertise of the scientists, technicians, practitioners, and sector professionals who continuously advance knowledge and translate research into practice.

These abstract proceedings reflect that collective expertise. They present current research and insights spanning milk extraction and milking systems, udder and teat hygiene, milk quality, and the prevention, detection, and treatment of mastitis, alongside broader aspects of udder health and lactation. Together, they offer a comprehensive overview of ongoing scientific advances and practical innovations shaping the future of dairy production.

I would like to express sincere appreciation to the conference organizers, sponsors, and all contributors whose dedication has made the IDF Mastitis and Milking Technology Joint Conferences 2026 possible. Their support and engagement are instrumental in fostering meaningful scientific exchange and collaboration.

I wish you an insightful and rewarding reading of these proceedings.

Laurence Rycken

IDF Director General

Welcome Message from the Organizers

Welcome to the IDF Mastitis and Milking Technology Joint Conference 2026.

It is our great pleasure to welcome you to this international gathering taking place in the beautiful city of Stockholm, Sweden, from 10–13 March 2026 at the Stockholm Waterfront Congress Centre.

This joint conference brings together members of the global dairy community to explore the latest scientific advances and practical innovations in milking technology, lactation biology, udder health, and mastitis management. Over the course of three days, experts from around the world will share new research findings, exchange knowledge, and engage in discussions that contribute to shaping the future of sustainable and efficient dairy production.

The conference provides a dynamic platform where researchers, veterinarians, industry leaders, advisors, and dairy farmers can exchange ideas, build collaborations, and strengthen the connection between science and practice. By bringing together diverse expertise and perspectives, we aim to foster meaningful dialogue and promote solutions that support animal health, milk quality, and the long-term sustainability of dairy farming systems. Beyond the scientific sessions, we encourage participants to take the opportunity to connect with colleagues from across the world and experience the welcoming atmosphere and hospitality of Sweden. We hope that every participant finds the conference in Stockholm inspiring, engaging, and full of meaningful connections.

We are delighted to have you join us and wish you a stimulating and collaborative conference experience in Stockholm.

Warm regards,
The Conference Organizing Committee
IDF Mastitis and Milking Technology Joint Conference 2026

Dr. Hasitha Priyashantha

Swedish National Committee Secretary of IDF
Milk Quality Expert at the Federation of Swedish Farmers (LRF Dairy), Stockholm, Sweden

Senior Lecturer
Department of Animal Science,
Faculty of Agriculture, University of Ruhuna,
Sri Lanka.

Co-Lead, Organizing and Scientific Committee

Dr. Carl Oskar Paulrud

Director, Dairy Development at DeLaval, Sweden

Co-Lead, Organizing and Scientific Committee

Prof. Sigrid Agenäs

Professor in management of ruminants
with a special focus on lactation biology

Director of the collaborative research centre
SustAinimal

Department of Applied Animal Science and
Welfare, Swedish University of Agricultural
Sciences, Uppsala, Sweden

Co-Lead, Organizing and Scientific Committee

Assoc. Prof. Monika Johansson

Swedish National Committee Secretary of IDF

Milk Quality Expert at the Federation of Swedish Farmers (LRF Dairy), Stockholm, Sweden

Co-Lead and Organizing

Editorial

IDF Mastitis and Milking Technology Joint Conference, Stockholm, 10–13 March 2026

The 2026 edition of the International Dairy Federation (IDF) Mastitis and Milking Technology Joint Conference brings together science, innovation, and practice at a time of rapid structural and technological change in the global dairy sector. Hosted in Stockholm, this joint platform reflects the growing recognition that udder health, milk harvesting technology, sustainability, and farmer decision-making are inseparably linked in shaping the future of dairy production.

Bovine mastitis remains one of the most economically consequential and biologically complex diseases in dairy production systems worldwide. It adversely affects milk yield, milk quality, animal welfare, antimicrobial usage, and overall farm profitability. While traditional diagnostic approaches such as clinical examination, somatic cell count measurement, and bacteriological culture have served as the foundation of mastitis control for decades, they are inherently occasional and often detect disease after inflammatory processes are well established. Addressing these multifaceted challenges requires not only improved diagnostic and management tools but also a broader understanding of the economic, technological, and biological contexts in which dairy production operates.

Against this backdrop, the scientific program opens with a forward-looking perspective on the global dairy business, setting the economic and policy context within which mastitis control and milking innovations must operate. This is complemented by expert insights into the biological–technological interface of milk harvesting, highlighting how advances in milking systems can be optimized to align with bovine physiology and welfare. Sessions on tailoring lactations to reduce health risks and on the cumulative impact of recurring mastitis underscore the long-term biological consequences of management decisions, bridging fundamental science with herd-level implementation.

A Strong and Diverse Scientific Contribution

This year's abstract proceedings demonstrate both scientific rigor and global engagement. Contributions originate from around 20 countries, with strong representation from Nordic and European dairy systems alongside research from North and South America, Australia, Asia and Central Asia (Figure 1). This multinational participation reflects the shared global challenge of mastitis control and the pursuit of sustainable milk production across diverse dairy production environments.

Figure 1. Geographical distribution of authors contributing to abstracts submitted to the IDF Mastitis and Milking Technology Joint Conference 2026, determined from the affiliations of all authors listed in the abstracts.

A clear majority of submissions employ quantitative methodologies, relying on robust statistical modeling, randomized clinical trials, longitudinal herd monitoring, and advanced laboratory diagnostics. Experimental and study designs are well balanced, reflecting a productive integration of hypothesis-driven interventions and real-world herd data analysis. Importantly, the program also includes mixed-methods research that integrates farmer interviews and behavioral insights with national register data, an encouraging sign that mastitis management is increasingly viewed through both biological and socio-economic perspectives. Such interdisciplinary approaches are particularly valuable in addressing complex herd health challenges, where technical solutions must be accompanied by effective adoption and management at the farm level.

Field- and farm-based studies slightly outnumber laboratory investigations, demonstrating the sector's commitment to translating research into practical on-farm solutions. At the same time, cutting-edge laboratory work, ranging from molecular diagnostics and proteomics to in vitro pathogen inhibition studies illustrates continued innovation in understanding host–pathogen interactions and improving diagnostic precision. Together, these complementary research approaches strengthen the evidence base required to advance mastitis prevention, early detection, and sustainable udder health management in modern dairy systems.

Emerging and Consolidated Research Themes

Five dominant themes emerge from the 2026 proceedings as follows,

1. **Udder Health Management and Mastitis Control**
   Research continues to refine the use of somatic cell counts, pathogen-specific monitoring, and microbiota analysis to enhance early detection and targeted intervention.

2. **Antimicrobial Stewardship and Sustainable Alternatives**
   Selective dry cow therapy, antimicrobial usage optimization, and non-antibiotic strategies, such as probiotics, integration of ethnoveterinary practices and omics-based approaches highlight the sector's proactive response to antimicrobial resistance (AMR).

3. **Advanced Diagnostics and Digital Integration**
   Rapid molecular tools, sensor technologies, automatic milking systems (AMS), and real-time data analytics are reshaping surveillance and treatment decision-making at herd level.

4. **Farmer Decision-Making and Adoption Science**
   Surveys and triangulated methodologies emphasize that successful mastitis control depends not only on technical solutions but also on farmer knowledge, attitudes, and behavioral drivers.

5. **Milking Equipment Optimization and Efficiency**
   Studies evaluating vacuum settings, cluster removal systems, hygiene protocols, and milk flow matching reflect continued efforts to optimize machine, animal and milker interactions.

Bridging Biology, Technology, and Sustainability

The 2026 conference highlights a clear paradigm shift. Mastitis research is no longer confined to pathogen identification and treatment efficacy. It now operates within an integrated systems framework encompassing animal welfare, antimicrobial stewardship, digitalization, farmer behavior, extension activies and economic sustainability.

By convening researchers, veterinarians, advisors, industry leaders, and policymakers, the IDF Joint Conferences provide a vital forum for translating science into actionable strategies. The strong emphasis on experimental validation, combined with real-world herd monitoring and socio-economic analysis, ensures that innovation remains grounded in practical relevance. As the dairy sector faces increasing pressure to enhance productivity while reducing environmental impact and antimicrobial reliance, the insights presented in Stockholm will contribute meaningfully to shaping resilient, science-based dairy systems worldwide.

The diversity, depth, and quality of the research presented in this abstract volume reflect the dynamism of the global mastitis and milking technology community. We extend our sincere appreciation to all authors, session chairs, and organizing partners for their valuable contributions. A special thanks goes to our reviewers, who generously shared their time, knowledge, and thoughtful feedback. Your careful evaluations and constructive insights have been instrumental in maintaining the high quality of the abstracts and ensuring that the conference reflects both scientific rigor and practical relevance. We are deeply grateful to all thse contributions made the IDF Mastitis and Milking Technology Joint Conference 2026 possible.

We wish all participants a stimulating and collaborative conference experience in Stockholm and hope that the conference will be inspiring, engaging, and foster many meaningful connections

With appreciation,

Editors

Dr. Hasitha Priyashantha, Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, 81100, Sri Lanka

Dr. John Upton, Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 C997, Ireland

Reviewer Recognition

We would like to warmly acknowledge the experts who generously contributed their time and expertise in reviewing the abstracts for this conference. Your efforts have been invaluable in shaping a strong, diverse, and high-quality scientific program.

1. Rupert Bruckmaier, Vetsuisse Faculty, University of Bern, **Switzerland**
2. Josef Dahlberg, , Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, **Sweden**
3. Sabine Ferneborg, Department of Animal and Aquaculture Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, **Norway**
4. Åsa Lundberg, Växa extension services, **Sweden**
5. Päivi Rajala-Schultz, Departments of Faculty of Veterinary Medicine, University of Helsinki, **Finland**
6. Doug Reineman, Biological Systems Engineering, College of Agricultural and Life SciencesUniversity of Wisconsin-Madison, **United States**
7. Dinah Seligsohn, Swedish Veterinary Agency, **Sweden**

We sincerely thank all reviewers for their thoughtful guidance and generous support for their contributions to strengthen this conference.

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IDF MASTITIS AND MILKING TECHNOLOGY JOINT CONFERENCE

11-13 MARCH, 2026 | STOCKHOLM

PROGRAM

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March 11

General Opening Session
Venue: Auditorium
10:00 – 12:00
Chair: *Sigrid Agenäs, Swedish University of Agricultural Sciences, Sweden*

10:00 – 10:30
Registration, posters

10:30
Opening and welcome
Laurence Rycken - IDF Director General and conference hosts

Invited talk:
Facts and Forecast of the global dairy business
Lynda McDonald, University of Adelaide & University of Nottingham Alliance, Joint PhD Program, New Zealand

Invited talk:
Contribution of milk harvesting research to optimal interaction between biology and milking technology
John Upton, Teagasc, Ireland

Discussion

12:00 – 13:30 **Lunch and posters**

13:30 – 15:00 **Parallel sessions**

Venue: A2
Mastitis theme session A1

13:30-15:00
Venue: A2
Mastitis theme session A1
Responsible Use of Antimicrobials for udder health
Chair: *Päivi Rajala-Schultz, University of Helsinki, Finland.*

13:30
Invited talk:
Prudent use if antibiotics in bovine mastitis. The Swedish approach.
Karin Persson Waller and Ylva Persson, Swedish Veterinary Agency.

14:02
Invited talk:
Rapid on-farm diagnosis of mastitis pathogens for clinical mastitis treatment decisions: update from South America
Marcos Veiga dos Santos, University of Sao Paolo, Brazil.

14:30
Selective Quarter-Based Antibiotic Dry Cow Therapy Assessing a Simplified Treatment Protocol Compared to Systematic Treatment.
Thomas Le Page, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, J2S 2M2, Québec, Canada.

14:40.
Omitting treatment of minor pathogens in quarter-selective dry cow therapy: Antibiotic consumption and cure rates.
Alexandra Beckmann, Institute of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany

14:50
Impact of selective treatment of non-severe clinical mastitis via an on-practice culture approach on 34 commercial dairy farms in Flanders, Belgium
Sofie Pipers, Ghent University and MEX™

15:00
The Antimicrobial Usage Practices of Michigan and Wisconsin Dairy Veterinarians Regarding the Treatment and Prevention of Mastitis.
Jaimie Strickland, Michigan State University1, East Lansing, United States.

15:10
Fika starts

Venue: A4
Milking technology theme session B1

13:30-15:00
Venue: A4
Milking technology theme session B1
Best practice milking
Chair: *Carl Oskar Paulrud*

13:30
Invited talk:
Modern dairy cow milking physiology
Rupert Bruckmaier, Prof. emeritus Dr. at the Vetsuisse Faculty, University of Bern, CH

14:15
Invited talk:
Novel technology meets the modern cow
Doug Reinemann, Professor of biological systems engineering, University of Wisconsin, Madison, USA

15:00
Fika starts

March 11

15:00 – 15:40	Venue: M1 Swedish fika (coffee break) and posters

Main session:
Changing landscapes of milk production and the milk value chain.
Venue: Auditorium
15:40-17:00
Chair: *Lynda McDonald, University of Adelaide & University of Nottingham Alliance, Joint PhD Program, New Zealand*

Invited talk:
Changing landscapes of milk value chain and milk production
Torsten Hemme, Researcher & entrepreneur; founder of IFCN, Germany

Invited talk:
Evolving the landscape of milk harvesting: Technology and innovation
Paul Löfgren, President & CEO of DeLaval

Invited talk:
Evolving the Food Production Landscape
Marie Sandin MD Tetra Pak Sweden & VP Global Strategic Programs

17:00 – 17:30	Venue: M1 Posters and mingle
18:30	Buses departure to the Gala Dinner at Berns
19:00 – 22:30	Gala Dinner at Berns and Entertainment
23:30 – 23:45	Buses from Berns to Waterfront

March 12

Main Session
Venue: Auditorium
09:00-10:00
Possibilities to manage milk yield and udder health through tailored lactation management and breeding
Chairperson: Anna Edvardsson Rasmussen, Sweden

Invited talk:
Possibilities to reduce health risks by tailoring dairy cow lactations
Ariette van Knegsel (WUR; The Netherlands

Invited talk:
Recurring mastitis – cumulative and lasting effect on mammary glands and milk
Angela Costa (University of Bologna, Italy)

10:00 – 10:40	**Venue: M1** **Swedish fika and posters**
10:40 – 12:00	**Parallel sessions**

Venue: A2
Mastitis theme session A2

10:40 – 12:00
New and Emerging Mastitis Threats
Chair: Josef Dahlberg, Sweden

10:40 – 11:10
Invited talk:
Got Flu? The H5N1 Enigma in U.S. Dairy Herds
Andrew S. Bowman (Ohio State University, US)

11:10 – 11:30
Invited talk:
Prototheca-mastitis outbreaks in three Finnish dairy herds
Leena Riipinen (Univ of Helsinki, Finland)

11:30
Addressing Diagnostic Challenges in Mycoplasma bovis Surveillance: A Pilot Study Using ELISA and PCR Bulk Tank Milk Samples
Lærke Boye Astrup, SEGES Innovation, Århus, Denmark.

11:40
Sensitivity in S. agalactiae surveillance on BMT and individual samples
Michael Farre, SEGES Innovation, Århus, Denmark.

11:50
Genetic diversity of Streptococcus agalactiae in Norwegian bovine dairy herds
Marit Smistad, TINE SA, Ås, Norway.

Venue: A4
Milking technology theme session B2

10:40 – 12:00
Milking system efficiency
Chair: Matthias Wieland

10:40 – 11:10
Invited talk:
Essential Concepts of Machine Milking
Ian Ohnstad (The Dairy Group UK)

11:10 – 11:40
Invited talk:
Teat cup removal and overmilking
Morten Dam Rasmussen, Senior Scientist, Aarhus University, Denmark

11:40 – 12:00
Invited talk:
Milk fat content at the end of milking as an indicator of the degree of udder emptying
Sara Almlöf, Cand Agro, MSc, PhD student, Swedish University of Agricultural Sciences

11:30
Addressing Diagnostic Challenges in Mycoplasma bovis Surveillance: A Pilot Study Using ELISA and PCR Bulk Tank Milk Samples
Lærke Boye Astrup, SEGES Innovation, Århus, Denmark.

March 12

12:00 – 13:30 | Venue: M1
Lunch and posters

13:30 – 15:00 **Parallel sessions**

Venue: A2
Mastitis theme session A3

13:30 – 15:00
Mastitis detection and treatment strategies
Invited talk:
Treatment of mastitis – progress made in the past.
Linda L. Tikofsky, private consultant, US
Panel discussion: The great mastitis debate – where are we at today?
Chaired by: Thomas Manske, Boehringer Ingelheim and Ellinor Eineren, AgriCam, Sweden

Venue: A4
Milking technology theme session B3

13:30 – 15:00
Milking system performance indicators
Chair: Morten Dam Rasmussen

13:30 – 14:15
Invited talk:
Performance and milk quality indicators at conventional farms
Matthias Wieland, DVM, Assistant Professor, Cornell University US

14:15 – 14:30
Oral presentation:
Matching milking settings to milk flow in a grazing system
Martin Browne, Teagasc, Ireland

14:30 – 14:45
Oral presentation:
Perceptions Drive Decisions How Farmers Understanding and use of Technology Shape Mastitis Management in Automatic Milking Systems
Dorota Anglart, PhD, Senior Researcher, DeLaval International AB, Adjunct Researcher, Swedish University of Agricultural Sciences, Sweden

14:45 – 15:00
Oral presentation:
Effective pre-milking hygiene protocols will contribute to reduce contamination of the milking equipment, milk and cows
Sofie Piepers, CEO MEX™, DMV, Professor Universiteit Gent, Belgium

15:00 – 15:40 | Venue: M1
Swedish fika and posters

15:40 – 17:00 **Parallel sessions**

Venue: A2
Mastitis theme session A3 continued

15:40 – 17:00
Mastitis detection and treatment strategies
Invited talk: **Udder health issues in the future – where are we going?**
Henk Hogeveen, WUR, The Netherlands
Panel discussion: Knowledge gaps and other bottle necks for future excellent udder health

Venue: A4
Milking technology theme session B4

15:40 – 17:00
Milking system performance indicators
Chair: Ian Ohnstad

15:40 – 16:15
Sponsored talk:
Milking time testing methods and key performance indicators in different milking systems
Tom Greenham, BVSc DBR MRCVS, Director, Advance Milking, UK

16:15 – 16:40
Invited talk:
Decades of successful Milking-Time Testing in the Nordic countries
Kaj Nyman, Dev. Manager, milking and milk cooling, Primary production, Valio Ltd, Finland

16:40 – 16:55
Oral presentation
Long Term Impact of Delayed Milk Ejection on Milk Yield
Paola Bacigalupo Sanguesa, DVM MS PhD, Michigan State University, US

March 12

17:10 – 18:20	**Short orals on how to Find and Fix Mastitis**

Venue: A2
Mastitis theme session A4
Chair: Sofie Piepers, Ghent University and MEX™

17:10
Potential of sensor systems monitoring animal health to support treatment decisions in clinical mastitis
Karin Knappstein, Institute of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany.

17:20
Frequent online cell count measurements enables and motivates farmers and advisors to work proactively with udder health
John Christensen, Lattec I/S, Hillerod, Denmark.

17:30
Addressing antimicrobial resistance A case study on the receptivity of Australian dairy farmers to a novel alternative treatment for bovine mastitis
Tiarna Scerri, Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW 2650, Australia.

17:40
Quantitative assessment of the antimicrobial activity of Lactococcus lactis isolates against Staphylococcus aureus in co-culture
Silvia Beschi, M-teamUGent, Ghent University, Belgium

17:50
Potential of MALDI-TOF mass spectra to predict spa types and persistence of intramammary infections caused by Staphylococcus aureus
Mariana Fonseca, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe J2S 2M2, QC, Canada.

18:00
Real-Time PCR in Mastitis Diagnostics
Heidi Hiitio, Pinehill Goblin Oy, Nurmijärvi, Finland

19:00 – 23:00
Pre drink

Dinner at Stockholm Waterfront and an evening of entertainment filled with ABBA nostalgia.

The dinner is sponsored by DeLaval and TetraPak.

Sponsored

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March 13

Venue: A2
Mastitis theme session A5

09:00 – 09:30 Scientific session with three short oral presentations
Chair: *Karin Persson Waller, Swedish Veterinary Agency.*

09:00
Cross-sectional pilot field study on Streptococcus uberis's mastitis in the Tessin region, Switzerland
Alicia Romano, Agroscope, Bern, Switzerland.

09:10
Seasonal dynamics of bulk milk somatic cell count in Norwegian dairy goats.
Marit Smistad, TINE SA, Ås, Norway

09:20
Genetic typing and in-vitro adherence profiles of Pseudomonas aeruginosa isolates from intramammary infections and bulk tank milk origins
Marcos Munoz, Universidad de Concepcion, Chillan, Chile.

Venue A1
Closing session

Auditorium 09:45 – 11:30

Closing remarks: *Gilles Froment, Senior VP Lactalis Canada & IDF President*

Panel discussion:
Torsten Hemme, Researcher & entrepreneur; founder of IFCN, Germany
Marie Sandin MD Tetra Pak Sweden & VP Global Strategic Programs, Sweden
Elisabeth Hidén, VP CEJA, Cand Agro, MSc & Dairy Farmer, Sweden
Cecilia Bågenvik, VP Business Development DeLaval, Sweden
Matthias Wieland, DVM, Assistant Professor, Cornell University, USA
Sigrid Agenäs, DVM, Director of SustAinimal & Professor SLU, Sweden
Thomas Manske, Boehringer Ingelheim

Rewards & Roundup:
Chair: *Lynda McDonald, University of Adelaide & University of Nottingham Alliance, Joint PhD Program, NZ*

11:30 – 13:00	**Lunch and mingle**
13:30	**Buses depart from Waterfront**
13:30-16:30 **Technical Tour/s**	Sponsored DeLaval

A: Hamra Farm (DeLaval's research farm, Tumba)

This sponsored visit to Hamra Farm includes a scientific program related to DeLaval products, as well as a tour of the brand-new research barn.

Buses depart from Waterfront at 1:30 PM and will return to Waterfront at 4:30 PM. The bus will be back at the hotel at approximately 5:00 PM.

B: Lövsta Farm (Swedish University of Agricultural Sciences' research farm, Uppsala)

This tour to the university research facilities is sponsored by DeLaval. The tour includes a scientific program as well as a guided tour of the research facilities.

Buses depart from Waterfront at 1:30 PM and return at 4:30 PM. The bus will be back at the hotel at approximately 5:15 PM and will also make a stop at Arlanda Airport on the return journey.

16:30	**Buses depart, with stops at Waterfront and Arlanda**
17:00 – 17:30	**End of conference**

Table of Contents

MASTITIS THEMATIC AREA

Photo: Lisa Chröisty, SLU

Somatic cell count as a practical decision tool for udder health management in Norwegian dairy goats

Marit Smistad[1*], Ragnhild Aabøe Inglingstad[1] and Siv Skeie[2]

[1] TINE SA, Ås, Norway

[2] Norwegian University of Life Sciences, Ås, Norway

***Corresponding author's email: marit.smistad@tine.no**

Introduction

Mastitis, primarily caused by bacterial intramammary infections (IMI), is a major challenge in dairy goat production, affecting milk quality, animal welfare, and productivity. Somatic cell count (SCC) is widely used for screening subclinical mastitis, but in goats, SCC is strongly influenced by non-infectious factors such as parity, lactation stage, season, and milk yield. This complicates the interpretation of SCC as an indicator of udder health compared to dairy cows. This study addresses gaps in understanding the relative contributions of infectious and non-infectious factors to SCC variation in goats and aims to define adjusted SCC thresholds for improved detection and management of IMI, particularly those caused by *Staphylococcus (S.) aureus*.

Material & methods

A longitudinal study was conducted in nine Norwegian dairy goat herds with an average herd size of 96 lactating goats in 2022 and 2023. The farms reflected typical Norwegian goat milk production practices, with spring kidding, indoor housing during early lactation, grazing on mountain pastures throughout mid-lactation, and a return to housing in late lactation. All lactating goats were sampled up to nine times during one (n= 5 farms) or two (n=4 farms) lactations for SCC and bacteriology, with data on parity, milk yield, and lactation stage retrieved from national records. Additionally, we utilized routine bulk milk analyses (SCC, total bacterial count) collected every third day during the study period and analyzed bacteriological composition of bulk milk at each sampling. Mixed linear and logistic regression models were used to quantify the effects of IMI and non-infectious factors on SCC, and to define parity- and season-adjusted SCC thresholds for *S. aureus* detection. Goat nested within herd was included as random effect.

Results & Discussion

The most frequently identified bacteriology findings were various non-aureus staphylococci and mammaliicocci (NASM), with *S. epidermidis, S. caprae*, and *S. warneri* detected in 6%, 6%, and 4% of the samples, respectively. *S. aureus* was found in 3% of the udder halves. The period prevalence of *S. aureus* varied from 1 to 40% in the included herds. The distribution of udder pathogens observed in this study is consistent with findings from a nationwide survey of Norwegian dairy goat herds (Smistad et al., 2021)

The model estimates of the linear mixed models (Smistad et al., 2024) showed a significant effect of IMI on SCC when adjusting for the lactation stage, parity and milk yield (Figure 1). Intramammary infections caused by *S. aureus*, increasing parity and the pasture season (which correlates with lactation stage in Norway) were the categories with highest impact on SCC (Figure 1). The model explained 57% of variation in SCC.

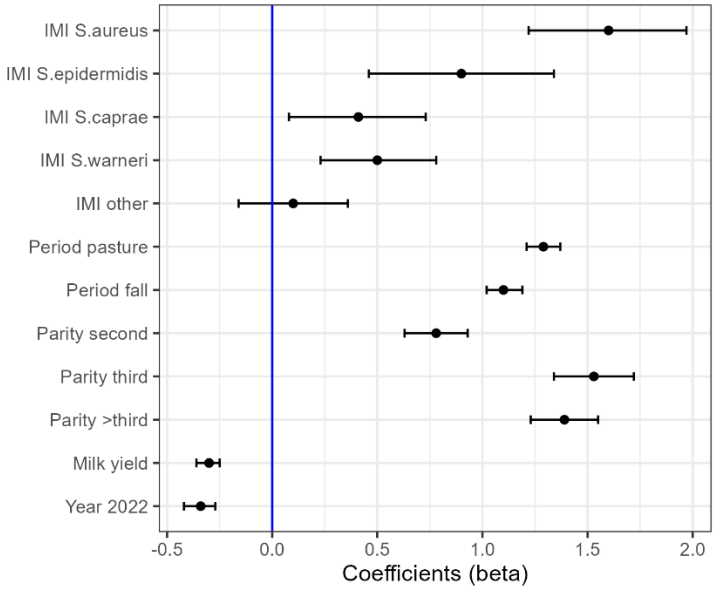


Figure 1: The estimated coefficients (β) and 95% confidence interval for associations (main effects) between ln-transformed somatic cell count (lnSCC), intramammary infection (IMI) status and non-infectious factors.

The mixed logistic models defined adjusted SCC thresholds for *S. aureus* detection (Smistad et al., 2025), and these thresholds varied considerably depending on parity, lactation stage, and pasture status: first parity goats in early lactation should be flagged at >500,000 cells/mL, while goats of higher parity on pasture may require thresholds up to 3 million cells/mL.

Bulk milk SCC and total bacterial count patterns, combined with bacteriological analyses of bulk milk, served as a practical and informative indicator of herd-level udder health status (Smistad et al., 2025). However, goat level milk recording samples for screening for SCC, followed by confirmation of IMI by bacteriology remains necessary.

Conclusion

Intramammary infections, particularly those caused by *S. aureus*, and non-infectious factors such as parity and pasture season, significantly influence SCC in Norwegian dairy goats. Adjusted SCC thresholds, considering parity and season, improve the identification of goats with IMI and support better udder health management. Bulk milk analyses are valuable for herd-level monitoring, but individual diagnostics are essential for targeted interventions. Future work should further refine SCC-based monitoring and explore the impact of management and stress factors on SCC in goats.

References

Smistad, M., Sølverød, L., Inglingstad, R. A., & Østerås, O. (2021). Distribution of somatic cell count and udder pathogens in Norwegian dairy goats. Journal of Dairy Science, 104(11), 11878–11888.

Smistad, M., Inglingstad, R. A., Sølverød, L., Skeie, S., & Hansen, B. G. (2024). Somatic cell count in dairy goats I: association with infectious and non-infectious factors. BMC Veterinary Research, 20(1), 509.

Smistad, M., Inglingstad, R. A., Vatne, M. K., Franklin, F. V., Hansen, B. G., Skeie, S., & Porcellato, D. (2025). Somatic cell count in dairy goats II: udder health monitoring at goat and herd level. BMC Veterinary Research, 21(1), 157.

Seasonal dynamics of bulk milk somatic cell count in Norwegian dairy goats

Marit Smistad[1*], Ragnhild Aabøe Inglingstad[1], Olav Østerås[1], and Siv Skeie[2]

[1] TINE SA, Ås, Norway

[2] Norwegian University of Life Sciences, Ås, Norway

***Corresponding author's email: marit.smistad@tine.no**

Introduction

Bulk milk somatic cell count (BMSCC) is a key indicator of udder health and milk quality in goat milk production. In Norway, goat farming is characterized by a seasonal, semi-extensive system that includes mountain pasture grazing during summer. This system contributes to a well-known annual fluctuation in BMSCC (Figure 1), recognized by farmers, advisors, and the dairy industry. To accommodate this variation, the Norwegian payment scheme for goat milk SCC is based on a 12-month rolling geometric average.

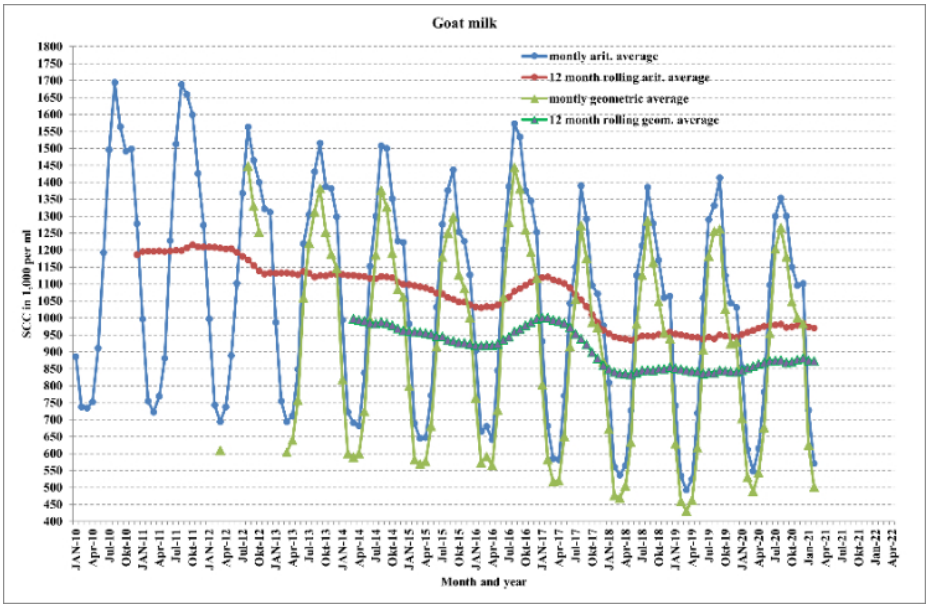


Figure 1: Bulk milk somatic cell count in Norwegian goat milk from 2010 to 2021, based on delivery data from ~250 farms annually. Data provided by TINE SA.

Despite the established seasonal pattern, the underlying causes of the summer rise in SCC remain poorly understood. Hypotheses include stress, increased physical activity, and elevated infection pressure during the pasture season. The GoatMilkSCC project was initiated to investigate these dynamics, refine the diagnostic utility of BMSCC for herd-level udder health surveillance, and assess its impact on product quality.

This study aimed to define seasonal BMSCC thresholds across production stages, explore the drivers of annual peaks, and evaluate associations between BMSCC and other milk quality parameters throughout lactation.

Material & methods

The study used two datasets: The first dataset included 5,180 routine bulk milk samples and questionnaire data from 88 Norwegian goat herds over one year (2021). The respondents represent approximately 40% of the Norwegian dairy goat farmers. With this data, we described the seasonal and lactational effects on BMSCC and its association with other milk quality parameters, and with data on management collected via questionnaire.

The second dataset included data from nine dairy goat herds from different regions of Norway through one lactation, combining routine BMSCC and total bacterial count (BMTBC) data from milk collections every third day. Individual goat intramammary infection status (bacterial culture) and SCC (DHI-recordings) were assessed at five sampling events in different lactation stages to address the contribution of intramammary infections (IMI) to the summer rise of BMSCC.

Results & Discussion

Smistad et al. (2024) reported median BMSCC (25^{th}, 75^{th} percentile) across seasons:
-Indoor early lactation (mean days in milk: 56): 470,000 (350,000, 620,000) cells/mL
-Pasture (summer, mean days in milk: 161): 1,100,000 (830,000, 1,470,000) cells/mL
-Mating period (mean days in milk: 212) 1,130,000 (830,000, 1,490,000) cells/mL
-Indoor late lactation (mean days in milk 243): 940,000 (700,000, 1,235,000) cells/mL
The increasing levels during lactation represent a combination of the seasonal effect (i.e. summer pasture) and later stages of lactation which include both reduced milk yield, estrus and pregnancy. With concentrated spring kidding, these effects are correlated in the Norwegian goat milk production and could not be disentangled in this data. Together, the season and stage of lactation explained 53% of the variation in BMSCC. BMTBC did not follow a seasonal pattern but showed a significant positive correlation with BMSCC during indoor seasons.

In the second dataset, no increase in IMI incidence was observed during the pasture season, despite a 3–5-fold rise in BMSCC (Smistad et al., 2025). This supports the hypothesis that the increase of BMSCC during summer is primarily of non-infectious

origin. Herds with a herd prevalence of *Staphylococcus aureus* IMI of <3% had BMSCC levels within the lower 25th percentile, while herds with >10% prevalence exceeded the 75th percentile of bulk milk thresholds provided in the first dataset. The herds with higher prevalence of IMI also showed unstable BMTBC, with several peaks >100,000 cells/mL (Smistad et al, 2025).

These findings suggest that the BMSCC levels identified in the first study may serve as practical benchmarks for farmers operating under similar management conditions. Specifically, values at or below the 25th percentile could be considered targets for high-performing herds, while levels exceeding the 75th percentile may indicate a need for improvement in udder health management. Importantly, these thresholds are not static throughout the year. Seasonal variation, particularly during the pasture season, was substantial, making cross-herd benchmarking less reliable during this period. Instead, herd-specific historical data should be used to establish action thresholds.

BMSCC increased immediately after pasture turnout in most herds, though magnitude varied. Few management factors were significantly associated with BMSCC rise (Steffensen and Heggheim, 2024). Herds with outdoor access outside grazing season showed less pronounced increases, highlighting the value of smoother indoor-outdoor transitions. Short-term BMSCC elevations were linked to stressors such as sudden weather changes, extreme walking distances, failure to return for milking, chasing by dogs etc. (Steffensen and Heggheim, 2024), consistent with findings from a controlled trial (Mehdid et al., 2019).

Conclusion

This study confirms the pronounced seasonal dynamics of BMSCC in Norwegian dairy goats and highlights the complexity of interpreting SCC in semi-extensive systems. The summer rise in BMSCC appears largely non-infectious, with stress and environmental transitions playing key roles. While especially *S. aureus* IMI contributes to elevated SCC and bacterial counts, its prevalence alone does not explain the seasonal pattern.

Season-specific benchmarks and herd-level historical data are essential for effective udder health monitoring. The findings support a nuanced approach to SCC interpretation, integrating seasonal context, infection status, and management practices. Future research should focus on controlled studies of stress-related SCC elevation and refinement of diagnostic tools for goat milk quality surveillance.

References

Smistad, M., Aab, R., & Skeie, S. (2024). Seasonal dynamics of bulk milk somatic cell count in grazing Norwegian dairy goats. JDS communications, 5(3), 205-209.

Smistad, M., Inglingstad, R. A., Vatne, M. K., Franklin, F. V., Hansen, B. G., Skeie, S., & Porcellato, D. (2025). Somatic cell count in dairy goats II: udder health monitoring at goat and herd level. BMC Veterinary Research, 21(1), 157.

Steffensen, I. and S. B. Heggheim. (2024). Non-infectious causes of elevated somatic cell count in dairy goats [in Norwegian]. Master thesis, Faculty of Veterinary Medicine, Norwegian University of Life Sciences

Mehdid, A., Martí-De Olives, A., Fernández, N., Rodríguez, M., & Peris, C. (2019). Effect of stress on somatic cell count and milk yield and composition in goats. Research in Veterinary Science, 125, 61-70.

Selective Quarter-Based Antibiotic Dry Cow Therapy: Assessing a Simplified Treatment Protocol Compared to Systematic Treatment

Thomas Le Page*, Simon Dufour and Jean-Philippe Roy
Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, J2S 2M2, Québec, Canada
Regroupement FRQNT Op+lait, Saint-Hyacinthe, J2S 2M2, Québec, Canada
***Corresponding author's email: Thomas.le.page@umontreal.ca**

Introduction

Selective antibiotic dry cow therapy (SDCT) is a proven method to reduce antimicrobial usage (AMU) at dry-off (McCubbin et al., 2023). Furthermore, meta-analyses have shown that the application of SDCT compared to blanket dry-cow therapy (BDCT) does not result in a poorer udder health (Kabera et al., 2021; Schipper et al., 2025). Moreover, further AMU reduction may be achievable through stricter cow or quarter selection, namely by combining a SCC-based selection with a subsequent bacteriology (Kabera et al., 2021). While these methods are more complex to use on a day-to-day basis, we previously determined that they do not significantly increase working time nor decrease the farm's gross margin (Le Page et al., 2024).

This study aims to evaluate, on various udder health criteria, a two-step in-series selection protocol—SCC screening followed by a quarter-specific on-farm rapid bacteriology (Petrifilm®, 3M)—compared to systematic antibiotic treatment of all quarters. We suppose that this method would reduce AMU by 90% while not affecting udder health.

Material & methods

A randomized control trial to compare our method and BDCT on five criteria representing udder health (and their respective clinical difference margin):

- Daily milk production (-1 kg/d),
- Clinical mastitis incidence in the first 120 days (+5%),

- Somatic Cell Score (SCS)(+0.5 units),
- New subclinical mastitis (SCM) rate (*i.e.* proportions of cows < 200 000 c/ml before dry-off and ≥200 000 cells/ml after calving; +5%),
- SCM cure rate (*i.e.* proportions of cows ≥ 200 000 c/ml before dry-off and < 200 000 cells/ml after calving; +5%).

We computed a sample size that was large enough to show if a clinical difference was present for any of these criteria with a 90% power (1-β), a minimum sample of 550 cows was required. We recruited 569 cows from 9 different farms. Cows were randomized to either: BDCT group (A-CTRL): all quarters of all cows received antimicrobials and teat sealant; Selective group (B-SDCT): only bacteriology positive quarters (Petrifilm®, 3M) from cows ≥200 000 cells/ml at last measurement received an antimicrobial, and all the quarters of all the cows received a teat sealant.

After treatment, we followed cows for 120 days. Criteria and AMU were used as outcomes in mixed models, with treatment group as a fixed effect, farm as a random effect, milk production at dry-off and lactation rank at dry-off were tested as interaction factors and kept in the model if significant (p<0.1). Estimated Marginal Means were then computed to allow a better understanding of the results.

Results & Discussion

At enrollment, A-CTRL and B-SDCT had a similar distribution among farms, SCC, daily milk production, lactation rank and days in milk. Table 1 presents the results of the five criteria representing udder health outcomes and AMU following the intervention.

Table 1: Results from a randomized controlled trial comparing a combined method of selective dry-cow therapy to blanket dry-cow therapy

Parameter	A-CTRL	B-SDCT	P-value
Daily milk production (kg/d)[1]	44.5 (40.9, 48.1)	44.8 (41.2, 48.4)	0.6
Somatic cell score[1]	1.8 (1.3, 2.2)	2.4 (2.0, 2.8)	<0.01
New SCM rate (%)[1]	9.0 (5.0, 15.4)	20.6 (14.0, 29.2)	<0.01
SCM cured rate (%)[2]	61	72	0.3
Clinical mastitis incidence in first 120 days (%)[1]	7.7 (4.7, 12.3)	9.9 (6.4 15.0)	0.3
Cow which received antimicrobials (%)[1]	99.6 (97.5, 99.9)	14.5 (9.95, 20.7)	<0.01
Quarters which received antimicrobials (%)[1]	99.7 (99.0, 99.9)	8.5 (5.24, 13.6)	<0.01

[1]Estimated Marginal Mean (confidence interval 95%), Fischer test; [2]Pearson's Chi square test
SCM: subclinical mastitis

Daily milk production, clinical mastitis and SCM cure rate did not differ between the two groups, while SCS was 0.6 points higher in group B-SDCT ($p<.01$) and new SCM rate was 11.6% higher in group B-SDCT. These results show a slightly worsening udder health due to SDCT, as SCS were slightly higher in that group. It seems that the increase in new SCM was mostly responsible for the increase post-calving SCS. A hypothesis is that some cows < 200 000 cells/ml before dry-off harbored pathogens but expressed no, or only a modest inflammation. At calving the infection persisted and the inflammation resumed. Quarter milk samples were collected for bacteriological analyses, which will help us understand this difference.

Cows in group B-SDCT received significantly less antimicrobial due to dry-cow therapy (-85%), and quarter received even less antimicrobials (-91%). This decrease in AMU was by far the largest reported in any randomized controlled trial on SDCT from literature More typically, decrease in AMU of 21 to 65% are observed (Kabera et al., 2020; Rowe et al., 2020; Vasquez et al., 2018).

Conclusion

These preliminary results suggest that our method of selective dry cow therapy massively reduces AMU at dry-off at the cost of a slight increase in SCC. Final analysis, including full bacteriology data, will clarify these findings and inform best practices for SDCT implementation.

References

Kabera, F., Dufour, S., Keefe, G., Cameron, M., & Roy, J.-P. (2020). Evaluation of quarter-based selective dry cow therapy using petrifilm on-farm milk culture : A randomized controlled trial. *Journal of Dairy Science*, *103*(8), 7276-7287. https://doi.org/10.3168/jds.2019-17438

Kabera, F., Roy, J.-P., Afifi, M., Godden, S., Stryhn, H., Sanchez, J., & Dufour, S. (2021). Comparing blanket vs. Selective dry cow treatment approaches for elimination and prevention of intramammary infections during the dry period : A systematic review and meta-analysis. *Frontiers in Veterinary Science*, *8*. https://doi.org/10.3389/fvets.2021.688450

Kabera, F., Roy, J.-P., Keefe, G., & Dufour, S. (2021). Bayesian estimation of diagnostic accuracy of somatic cell counts history and on-farm milk culture using Petrifilm® to identify quarters or cows that should be treated with antimicrobials in selective treatment protocols at dry off. *Preventive Veterinary Medicine*, *195*, 105452. https://doi.org/10.1016/j.prevetmed.2021.105452

Le Page, T., Ferchiou, A., Dufour, S., Kabera, F., Dubuc, J., Lhermie, G., Raboisson, D., & Roy, J.-P. (2024). Dairy farmer income, working time, and antimicrobial use under different dry cow therapy protocols. *Journal of Dairy Science*, *107*(10), 8115-8129. https://doi.org/10.3168/jds.2023-24407

McCubbin, K. D., Jong, E. de, Brummelhuis, C. M., Bodaneze, J., Biesheuvel, M., Kelton, D. F., Uyama, T., Dufour, S., Sanchez, J., Rizzo, D., Léger, D., & Barkema, H. W. (2023). Antimicrobial and teat sealant use and selection criteria at dry-off on Canadian dairy

farms. *Journal of Dairy Science*, *106*(10), 7104-7116. https://doi.org/10.3168/jds.2022-23083

Rowe, S., Godden, S., Nydam, D. V., Gorden, P., Lago, A., Vasquez, A., Royster, E., Timmerman, J., & Thomas, M. (2020). Evaluation of rapid culture, a predictive algorithm, esterase somatic cell count and lactate dehydrogenase to detect intramammary infection in quarters of dairy cows at dry-off. *Preventive Veterinary Medicine*, *179*, 104982. https://doi.org/10.1016/j.prevetmed.2020.104982

Schipper, N., Bodmer, M., Dufour, S., Hommels, N. M. C., Nielen, M., & van den Borne, B. H. P. (2025). Network meta-analysis based ranking of dry off interventions to cure or prevent intramammary infections in dairy cows. *Preventive Veterinary Medicine*, *239*, 106487. https://doi.org/10.1016/j.prevetmed.2025.106487

Vasquez, A. K., Nydam, D. V., Foditsch, C., Wieland, M., Lynch, R., Eicker, S., & Virkler, P. D. (2018). Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. *Journal of Dairy Science*, *101*(6), 5345-5361. https://doi.org/10.3168/jds.2017-13807

Impact of selective treatment of non-severe clinical mastitis via an on-practice culture approach on 34 commercial dairy farms in Flanders, Belgium

(On-practice culture selective treatment)

Creytens Lien [1*], Piepers Sofie[1], and De Vliegher Sarne[1]

[1] Ghent University, Merelbeke, Belgium

***Corresponding author's email: Lien.Creytens@UGent.be**

Introduction

Mastitis accounts for the majority of antimicrobial use (AMU) on dairy farms (Stevens et al., 2016). Blanket antimicrobial treatments at drying off or in clinical mastitis (CM) cases without pathogen identification have raised concerns regarding antimicrobial resistance and prudent AMU (Oliver et al., 2011). Selective dry cow therapy (SDCT) and selective treatment of non-severe CM (STCM) offer effective alternatives to reduce AMU without compromising udder health or production (McCubbin et al., 2022; de Jong et al., 2023). Previous research on STCM has mainly focused on "on-farm" testing, where producers perform and interpret rapid testing systems, a method requiring sufficient training and experience (Lago et al., 2011; Bates et al., 2020). In contrast, "on-practice" testing is conducted by veterinary practices (VP). The latter offers professional interpretation and may enhance responsible AMU, particularly in regions with smaller, closely located dairy herds such as Flanders. To evaluate the effects of STCM through an on-practice culture approach, a two-year randomized clinical field trial was conducted on commercial Flemish dairy farms supported by veterinary practices. The study compared somatic cell count (SCC), milk production (MP), AMU, bacteriological and clinical cure, and milk withdrawal time between an immediate and a culture-based treatment protocol.

Material & methods

A two-year randomized clinical field trial was conducted across 34 commercial Flemish dairy farms supported by 10 VPs to evaluate STCM. Participating VPs were trained to perform and interpret on-practice rapid tests (RT) for pathogen detection in milk and to provide antimicrobial treatment (AMT) advice. Dairy producers enrolled cows with non-

severe clinical mastitis and randomly assigned cases to either an immediate treatment protocol (IT) (immediate AMT at detection) or a culture-based treatment protocol (CBT), in which AMT was guided by RT outcomes. In the CBT group, AMT was only initiated for gram-positive infections or contaminated samples, whereas gram-negative or culture-negative results received no AMT. All producers recorded clinical signs, treatment (intramammary and systemic AMT and NSAID's), and milk withdrawal data daily. Quarter milk samples were collected at detection (D0) and at day 21 for bacteriological culture to assess bacteriological cure (BC). Milk production and SCC were monitored for 6 subsequent test-day recordings.

Outcome variables included antimicrobial treatment incidence (ATI), days until clinical cure, milk withdrawal days, BC, natural logarithm of SCC, and MP. Statistical analyses were performed using SAS® OnDemand for Academics version 3.82 (SAS Institute Inc., Cary, NC, US) and SPSS® (version 26.0; SPSS Inc., Chicago, IL, US). Non-parametric independent-samples Mann–Whitney U tests were applied to non-normally distributed data, and mixed logistic and linear multivariable models accounted for clustering within herds and veterinary practices. Results were seen as significant when $P<0.05$.

Results & Discussion

A total of 318 quarter cases (157 and 161 in IT and CBT, respectively) were enrolled. Herds differed in size and management systems but were comparable in SCC, MP, days in milk, and parity at enrollment. Implementation of selective treatment via the CBT-protocol resulted in a 43% reduction in cows treated with antimicrobials and a significantly lower ATI (median and interquartile range for CBT- and IT-protocol are 26 and 40.70 DDDA per 1,000 cow days and 62.34 and 45.60 DDDA per 1,000 cow days, respectively; $P = 0.005$). Days to clinical cure did not differ between protocols, although treated cases in both protocols showed a marginally longer recovery time. Bacteriological cure was numerically lower in CBT (64%) than in IT (78%), but differences were not statistically significant. Milk withdrawal time was significantly reduced in CBT (6.2 vs. 8.9 days; $P < 0.001$), and no significant effects were observed for SCC or MP.
Variability in pathogen distribution and management practices among the participating farms supports the external validity of these findings and suggests that similar AMU reductions are achievable under diverse farm conditions (Verbeke et al., 2014; Stevens et al., 2016). It must be pointed out that the amount of AMU reduction depends on the herd-specific pathogen profile and udder health management and on the characteristics of the used RTs (Lago et al., 2011, Bates et al., 2020; Ruegg, 2025).

Conclusion

Our findings indicate that STCM based on on-practice culture results effectively decreases AMU and milk withdrawal time, without adverse effects on udder health or productivity. Integrating veterinary guidance within this framework supports prudent antimicrobial stewardship and enhances the overall sustainability of mastitis management.

References

Bates, A., Laven, R., Bork, O., Hay, M., McDowell, J., & Saldias, B. (2020). Selective and deferred treatment of clinical mastitis in seven New Zealand dairy herds. *Preventive veterinary medicine, 176,* 104915.

de Jong, E., Creytens, L., De Vliegher, S., McCubbin, K.D., Baptiste, M., Leung, A.A., Speksnijder, D., Dufour, S., Middleton, J.R., Ruegg, P.L., Lam, T.J.G.M., Kelton, D.F., McDougall, S., Godden, S.M., Lago, A., Rajala-Schultz, P.J., Orsel, K., Krömker, V., Kastelic, J.P., & Barkema, H.W. (2023). Selective treatment of non-severe clinical mastitis does not adversely affect cure, somatic cell count, milk yield, recurrence, or culling: A systematic review and meta-analysis. *Journal of Dairy Science, 106,* 1267–1286.

Lago, A., Godden, S.M., Bey, R. , Ruegg, P.L., & Leslie, L. (2011). The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. *Journal of Dairy Science, 94*, 4441-4456.

McCubbin, K., de Jong, E. , Lam, T.J.G.M., Kelton, D.F., Middleton, J.R., McDougall, S., De Vliegher, S., Godden, S., Rajala-Schultz, P.J., Rowe, S., Speksnijder, D.C., Kastelic, J.P., & Barkema, H.W. (2022). Invited review: Selective use of antimicrobials in dairy cattle at drying-off. *Journal of Dairy Science, 105,* 7161-7189. https://doi.org/10.3168/jds.2021-21455

Oliver, S., Murinda, S., & Jayarao, B. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. *Foodborne Pathogens and Disease, 8(3),* 337-55.

Ruegg, P.L. (2025). The future of udder health: antimicrobial stewardship and alternative therapy of bovine mastitis. *Journal of Dairy Science Communications* (in press) https://doi.org/10.3168/jdsc.2025-0839

Stevens, M., Piepers, S., & De Vliegher, S. (2016). Mastitis prevention and control practices and mastitis treatment strategies associated with the consumption of (critically important) antimicrobials on dairy herds in Flanders, Belgium. *Journal of Dairy Science, 99*, 2896-2903.

Verbeke, J., Piepers, S., Supré, K., & De Vliegher, S. (2014). Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene. *Journal of Dairy Science, 97(11), 6926-6934*.

Potential of MALDI-TOF mass spectra to predict spa types and persistence of intramammary infections caused by Staphylococcus aureus

Mariana Fonseca[1,2*], Jean-Philippe Roy[2,3], Anitza Fragas Quintero[2,4], François Malouin[2,4], and Simon Dufour[1,2]

[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe J2S 2M2, QC, Canada

[2]Regroupement FRQNT Op+lait, Saint-Hyacinthe J2S 2M2, QC, Canada

[3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe J2S 2M2, QC, Canada

[4]Departement de Biologie, Faculté des sciences, Université de Sherbrooke, Sherbrooke J1K 2R1, QC, Canada

***Corresponding author: m.fonseca@umontreal.ca**

Introduction

Staphylococcus aureus is a leading cause of intramammary infections (IMI) in Canadian dairy herds and is frequently isolated from both clinical mastitis cases and apparently healthy cows (Reyher et al., 2011). These infections are commonly associated with elevated somatic cell count (SCC) (Franzoi et al., 2020), contributing to economic losses due to reduced milk production and quality (Aghamohammadi et al., 2018). Persistent IMI caused by *S. aureus* are of particular concern, as persistent strains often exhibit genotypic differences from non-persistent ones, differences likely related to virulence factors promoting immune evasion and long-term colonization. In contrast, non-persistent strains may display a more acute virulence profile, marked by higher cytotoxin expression and rapid immune clearance. Due to its reproducibility and discriminatory power, *spa* typing is widely employed in epidemiological investigations to track the transmission of *S. aureus* strains within and between herds, and to explore associations with virulence factors. In a Canadian study, Pichette-Jolette et al. (2019) used *spa* typing to identify *S. aureus* isolates and found that *spa* type t529 (the most prevalent) was significantly less associated with persistence. Strains of this type were less likely to produce biofilm (*vs* hyperproducers like t13401 and t605) and 97% carried

the superantigen genes *sen* and *seg*, which may reflect a distinct virulence strategy more compatible with acute infection rather than persistence. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has transformed microbial identification and has shown potential for detecting strain-level traits such as virulence factors (Bizzini & Greub, 2010). Therefore, the objectives of this study were: (1) to evaluate the performance of machine learning algorithms in predicting the six most common *spa* types of *S. aureus* isolates from Canadian dairy farms based solely on MALDI-TOF mass spectra; and (2) to assess whether these algorithms could distinguish between persistent and short-term IMI caused by *S. aureus*.

Material & methods

As part of the National Cohort of Dairy Farms, milk samples were collected weekly from 15 randomly selected lactating cows in 91 Canadian herds between 2007 and 2008 (Reyher et al., 2011). For the first objective, 373 *S. aureus* isolates that had previously undergone *spa* typing, as described by Pichette-Jolette et al. (2019), were select for further analysis. For the second objective, 1,602 quarter milk samples with at least one *S. aureus* isolation were selected. To classify infection duration, the results from milk samples collected on a given mammary quarter were organized longitudinally, with persistent IMI defined as ≥5 *S. aureus*-positive samples per quarter during the sampling period, and short- term IMI defined as a single *S. aureus*-positive sample preceded and followed by two consecutive negative samples. A total of 370 quarters met these case definitions. For persistent IMI, the first *S. aureus* isolate in the series was selected, while for short-term IMI, the isolate detected between the negative samples was included (222 persistent, 148 short-term). All isolates from both objectives were analyzed by MALDI-TOF MS, and the resulting spectra were preprocessed before analysis (smoothing, baseline correction, intensity calibration, and alignment). Reference peaks were identified from the training set and used to guide feature extraction from the validation dataset. Feature matrices were built using the reference peaks and their corresponding intensity values. Random forest models were then trained for classification, and their performance was assessed using the area under the receiver operating characteristic curve (ROC-AUC), sensitivity, and specificity.

Results & Discussion

When used for distinguishing intrinsic characteristics of *S. aureus*, MALDI-TOF MS has shown very promising results. For instance, Jeon et al. (2022) have demonstrated its ability to identify specific spectral peaks that can classify *S. aureus* isolates as either methicillin susceptible or resistant with an accuracy of 88%. For the first objective, our models demonstrated strong classification performance for *spa* types t529, t605, and t3401, with AUCs of 0.88, 0.94, and 0.89, respectively. The mean sensitivity (95% CI) and specificity (95% CI) were: 0.82 (0.72–0.90) and 0.94 (0.87–0.98) for t529; 0.88 (0.47–0.99) and 1.00 (0.98–1.00) for t605; and 0.80 (0.44–0.98) and 0.98 (0.95–1.00) for t3401. For the other three spa types, model performance was poor, with AUC values of 0.53, 0.68, and 0.62 for *spa* types t359, t267, and t2445, respectively. The average mass spectra for each *spa* type are presented in Figure 1. In contrast, for the second objective, the model for predicting IMI duration showed limited performance, with an AUC of 0.53. While sensitivity was high (99.2%, 95% CI: 95.5–100.0%), specificity was extremely low (0.06%, 95% CI: 0.02–0.1%).

Conclusion

These findings suggest that MALDI-TOF MS can reasonably predict certain *spa* types of *S. aureus*, highlighting its potential as a cost-effective and accessible alternative to

more resource-intensive molecular typing methods in epidemiological studies. However, its poor performance in distinguishing between persistent and short-term IMI underscores the challenge of relying solely on spectral features to infer clinical outcomes, likely due to the multifactorial nature of infection persistence, which involves complex host-pathogen interactions.

References

Aghamohammadi, M., Haine, D., Kelton, D. F., Barkema, H. W., Hogeveen, H., Keefe, G. P., & Dufour, S. (2018). Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. *Frontiers in Veterinary Science*, *5*, 100. https://doi.org/10.3389/fvets.2018.00100

Bizzini, A., & Greub, G. (2010). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clinical Microbiology and Infection*, *16*(11), 1614–1619. https://doi.org/10.1111/j.1469-0691.2010.03311.x

Franzoi, M., Manuelian, C. L., Penasa, M., & De Marchi, M. (2020). Effects of somatic cell score on milk yield and mid-infrared predicted composition and technological traits of Brown Swiss, Holstein Friesian, and Simmental cattle breeds. *Journal of Dairy Science*, *103*(1), 791–804.

Jeon, K., J.-M. Kim, K. Rho, S.H. Jung, H.S. Park, and J.-S. Kim. 2022. Performance of a Machine Learning-Based Methicillin Resistance of Staphylococcus aureus Identification System Using MALDI-TOF MS and Comparison of the Accuracy according to SCCmec Types. Microorganisms 10:1903. doi:10.3390/microorganisms10101903.

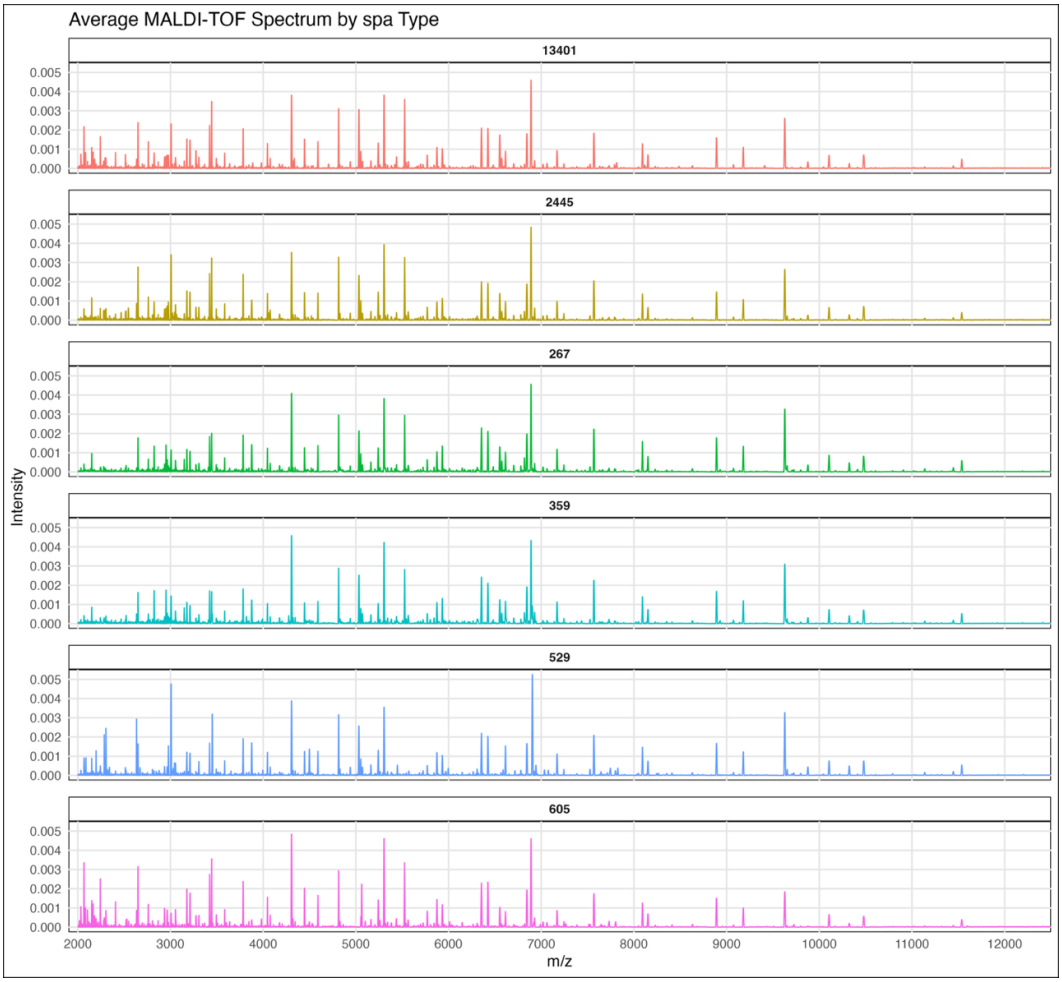
Reyher, K. K., Dufour, S., Barkema, H. W., Des Côteaux, L., DeVries, T. J., Dohoo, I. R., Keefe, G. P., Roy, J.-P., & Scholl, D. T. (2011). The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *Journal of Dairy Science*, *94*(3), 1616–1626. https://doi.org/10.3168/jds.2010-3180

Tuchscherr, L., Medina, E., Hussain, M., Völker, W., Heitmann, V., Niemann, S., Holzinger, D., Roth, J., Proctor, R. A., Becker, K., Peters, G., & Löffler, B. (2011). *Staphylococcus aureus* phenotype switching: An effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Molecular Medicine*, *3*(3), 129–141. https://doi.org/10.1002/emmm.201000115

Waters, E. M., Rowe, S. E., O'Gara, J. P., & Conlon, B. P. (2016). Convergence of Staphylococcus aureus Persister and Biofilm Research: Can Biofilms Be Defined as Communities of Adherent Persister Cells? *PLOS Pathogens*, *12*(12), e1006012.

Pichette-Jolette, S., G. Millette, E. Demontier, D. Bran-Barrera, M. Cyrenne, C. Ster, D. Haine, G. Keefe, F. Malouin, and J.P. Roy. 2019. Partial prediction of the duration and the clinical status of Staphylococcus aureus bovine intramammary infections based on the phenotypic and genotypic analysis of isolates. Veterinary Microbiology 228:188–195. doi:10.1016/j.vetmic.2018.11.024.

Figure 1. Preprocessed and averaged mass spectra for *S. aureus* isolates classified according to *spa* type.


Average MALDI-TOF Spectrum by spa Type
13401
2445
267
359
529
605
Intensity
0.005
0.004
0.003
0.002
0.001
0.000
2000
3000
4000
5000
6000
7000
8000
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Quantitative assessment of the antimicrobial activity of *Lactococcus lactis* isolates against *Staphylococcus aureus* in co-culture

Silvia Beschi*, Bruno Toledo-Silva, Kristien Mertens and Sarne De Vliegher
M-teamUGent, Ghent University, Belgium
***Corresponding author's email: silvia.beschi@ugent.be**

Introduction

Bovine mastitis, an inflammation of the udder, is one of the most detrimental diseases in dairy farming, reducing profitability and affecting animal welfare, and the dairy sector's image (Piepers & De Vliegher, 2018). It is mainly caused by bacterial infections, with *Staphylococcus aureus* representing a major contagious pathogen, often causing chronic mastitis; antibiotic-resistant strains further complicate treatment (Cheng & Han 2020). In the context of the global antimicrobial resistance crisis, reducing reliance on conventional antibiotic treatments on dairy herds has become urgent, prompting research into innovative and sustainable alternatives. One promising approach is the use of mammary probiotics for the prevention and treatment of mastitis (Kober, Saha, Islam, Rajoka, Fukuyama, Aso, Villena, & Kitazawa, 2022).

Among the bacterial species investigated, *Lactococcus lactis* has emerged as a promising candidate. Previous studies have demonstrated its antimicrobial activity against mastitis pathogens (Bouchard, Seridan, Saraoui, Rault, Germon, Gonzalez-Moreno, Nader-Macias, Baud, François, Chuat, Chain, Langella, Nicoli, Le Loir, & Even, 2015; Pellegrino, Frola, Natanael, Gobelli, Nader-Macias, & Bogni, 2019); however, most relied on assays at a single fixed concentration or on semiquantitative evaluations, which do not capture the extent and variability of the inhibitory effects among different *L. lactis* strains. Therefore, in this work we investigated, for the first time, the antimicrobial potential of *L. lactis* strains isolated from Belgian dairy farms against *S. aureus*, using a co-culture assay that enables quantitative and precise assessment of their inhibitory activity.

Material & methods

Isolation of *Lactococcus lactis*

Bulk tank milk samples were collected from 40 dairy farms in Belgium using sterile pipettes and syringes while the tank's agitator was operating. Samples were transported at 4°C to the Mastitis and Milk Quality Research Laboratory (Ghent University, Belgium) for analysis. To maximize *L. lactis* recovery, 1 mL of each sample was cultured on M17 agar and incubated anaerobically at 37 °C overnight. Phenotypically distinct colonies were subcultured on blood agar to obtain pure cultures. Catalase testing was performed, and all catalase-negative isolates were further identified using MALDI-TOF MS.

Co-culture assay

The co-culture assay was performed according to Soundharrajan et al. (Soundharrajan, Yoon, Muthusamy, Jung, Lee, Han, & Choi, 2021), with minor modifications. Briefly, *L. lactis* isolates and *S. aureus Newbould 305* strain were cultured in tryptic soy broth (TSB) until reaching approximately 10^8 CFU/mL. Bacterial suspensions were harvested by centrifugation at 3,200 × g for 10 min, washed twice with sterile phosphate-buffered saline (PBS), and resuspended in sterile TSB. Ten-fold serial dilutions were then prepared to obtain working suspensions.

For the co-culture assay, 100 µL of each *L. lactis* suspension (between 10^5 and 10^8 CFU/mL) was added to a 96-well plate, resulting in final inocula of 10^4- 10^7 CFU per well. Subsequently, 100 µL of *S. aureus* suspensions (10^2 - 10^6 CFU/mL) were added to each *L. lactis* suspension, in triplicate, resulting in final *S. aureus* inocula between 10^1 and 10^5 CFU per well. *L. lactis LMG 7930*, a characterized inhibitor (Armas, Camperio, & Marianelli, 2017), was included as a positive control in each assay. Negative controls consisted of 100 µL *S. aureus* dilutions mixed with equal volume of sterile TSB. Plates were incubated aerobically at 37 °C overnight.

Following incubation, the co-cultures were serially diluted in sterile PBS, and 10 µL of each dilution was spotted onto mannitol salt agar (MSA) plates. After 18 h of aerobic incubation at 37 °C, *S. aureus* growth was enumerated by counting CFU in droplets containing approximately 3–50 colonies. The inhibitory effect of *L. lactis* was expressed as log CFU reduction, calculated by comparing *S. aureus* counts in co-cultures with those in negative control wells containing *S. aureus* alone.

Results & Discussion

In total, 12 *L. lactis* isolates were recovered from Belgian dairy farms. At this stage, the co-culture assay has been optimized and tested with a single *L. lactis* randomly selected isolate. Preliminary results are promising and indicate a potential dose-related inhibitory effect of *L. lactis* on *S. aureus*. The strongest inhibition was observed when the highest concentration of the *L. lactis* isolate (10^7 CFU) was co-cultured with the lowest concentration of *S. aureus (*10^2 CFU), resulting in a 2.5-log reduction compared with control wells containing *S. aureus* alone. These preliminary results are consistent with previous studies reporting the ability of *L. lactis* to inhibit mastitis pathogens, including *S. aureus* (Bouchard et al., 2015; Pellegrino et al., 2019). Further experiments are underway to confirm these findings by repeating the assay with this specific *L. lactis* isolate and extending the analysis to the remaining *L. lactis* strains and more mastitis pathogens, including gram-negatives. Importantly, our approach of co-culturing different concentrations of *L. lactis* with varying inocula of *S. aureus* allows a more quantitative

assessment of the antimicrobial activity than conventional inhibition assays. This strategy will not only allow identification of the most effective inhibitory concentrations but also provides insights into intra- and inter-strain variability among *L. lactis* isolates in inhibitory activity, contributing to a deeper understanding of their protective potential against *S. aureus*.

Conclusion

This study aims to generate valuable insights into the potential use of *L. lactis* as a natural antimicrobial agent against *S. aureus*. By demonstrating quantitative evidence of *L. lactis in vitro* inhibitory activity, this work provides a solid foundation for further studies investigating their potential application in mastitis prevention. Ultimately, these findings may support the development of sustainable, probiotic-based strategies to reduce antibiotic reliance in dairy farming.

References

Armas, F., Camperio, C., & Marianelli, C. (2017). In Vitro Assessment of the Probiotic Potential of *Lactococcus lactis LMG 7930* against Ruminant Mastitis-Causing Pathogens. *PloS one*, *12*(1), e0169543.

Bouchard, D. S., Seridan, B., Saraoui, T., Rault, L., Germon, P., Gonzalez-Moreno, C., Nader-Macias, F. M., Baud, D., François, P., Chuat, V., Chain, F., Langella, P., Nicoli, J., Le Loir, Y., & Even, S. (2015). Lactic Acid Bacteria Isolated from Bovine Mammary Microbiota: Potential Allies against Bovine Mastitis. *PloS one*, *10*(12), e0144831.

Cheng, W. N., & Han, S. G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. *Asian-Australasian journal of animal sciences*, *33*(11), 1699–1713.

Kober, A. K. M. H., Saha, S., Islam, M. A., Rajoka, M. S. R., Fukuyama, K., Aso, H., Villena, J., & Kitazawa, H. (2022). Immunomodulatory Effects of Probiotics: A Novel Preventive Approach for the Control of Bovine Mastitis. *Microorganisms*, *10*(11), 2255.

Pellegrino, M. S., Frola, I. D., Natanael, B., Gobelli, D., Nader-Macias, M. E. F., & Bogni, C. I. (2019). In Vitro Characterization of Lactic Acid Bacteria Isolated from Bovine Milk as Potential Probiotic Strains to Prevent Bovine Mastitis. *Probiotics and antimicrobial proteins*, *11*(1), 74–84.

Piepers, S., & De Vliegher, S. (2018). Alternative approach to mastitis management – How to prevent and control mastitis without antibiotics?. Brazilian Journal of Veterinary Research and Animal Science, 55(3).

Soundharrajan, I., Yoon, Y. H., Muthusamy, K., Jung, J. S., Lee, H. J., Han, O. K., & Choi, K. C. (2021). Isolation of *Lactococcus lactis* from Whole Crop Rice and Determining Its Probiotic and Antimicrobial Properties towards Gastrointestinal Associated Bacteria. *Microorganisms*, *9*(12), 2513.

Addressing Diagnostic Challenges in *Mycoplasma bovis* Surveillance: A Pilot Study Using ELISA and PCR Bulk Tank Milk Samples

Lærke Boye Astrup[1*], Malene Vesterager Byskov[1], Erik Rattenborg[1] Anne Mette Hostrup Kjeldsen[1], Michael Farre[1]

[1] SEGES Innovation, Århus, Denmark

***Corresponding author's email: lbas@seges.dk**

Introduction

Mycoplasma bovis (*M. Bovis*) is a significant pathogen in dairy cattle, associated with mastitis, arthritis, pneumonia, and considerable economic losses. Effective herd-level surveillance is essential for early detection, control, and prevention of disease transmission.

In Denmark, the prevalence of *M. bovis* has been monitored through a national surveillance program since 2011, using PCR analysis of bulk tank milk (BTM) samples. BTM sampling offers a practical, non-invasive, and cost-effective method for assessing the infection status of entire milking herds. It also allows for broad and consistent coverage, as legislation mandates the collection of milk samples from all dairy herds supplying milk for human consumption in Denmark, facilitating routine and timely surveillance.

However, reliance on PCR alone presents challenges. Shedding *M. bovis* in milk is intermittent, reducing the sensitivity of PCR-based detection in BTM samples. In several cases, herd veterinarians have reported clinical signs consistent with *M. bovis* infection in herds that tested PCR-negative. Also, it is likely that some cows are being removed from the milking herd and producing waste milk not identified in BTM sampling. Furthermore, routine mastitis diagnostics in Denmark do not typically identify

Mycoplasma species, reinforcing the importance of a robust surveillance program based on BTM sampling.

Serological tools, such as ELISAs, offer a complementary approach by detecting antibodies that persist beyond the period of bacterial shedding. The ID Screen® *M. bovis* indirect ELISA (IDvet, France), which targets antibodies to a recombinant fragment of the MilA protein, has been widely used for BTM testing. However, its diagnostic sensitivity (DSe) and specificity (DSp) can vary depending on the epidemiological context and the population tested.

To support data-driven decision-making and enhance the utility of ELISA in surveillance, robust estimates of test performance characteristics—such as DSe, DSp, and the optimal cut-off are needed. This pilot study aimed to compare PCR and the ID Screen® *M. bovis* indirect ELISA, for identifying dairy herds positive for *M. Bovis*.

Material & methods

This study was designed as a cross-sectional survey to evaluate the diagnostic performance of an ELISA test for detecting *M. bovis* antibodies in bulk tank milk (BTM). The target population consisted of all commercial dairy herds in the country actively shipping milk for human consumption. At the time of sampling, this comprised a total of 2,215 herds.

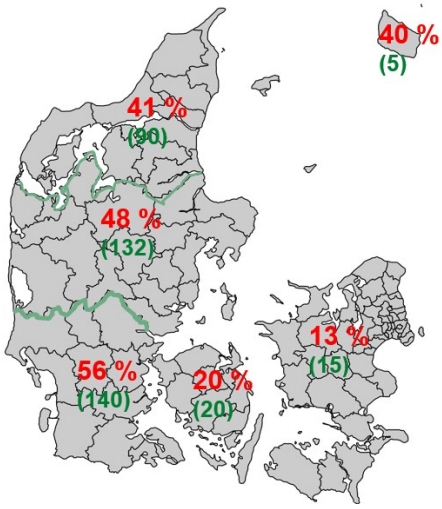
From this population, a sample of 402 herds was selected using simple random sampling without replacement. The sampling frame included all eligible dairy herds registered as shipping milk with a milk processor, ensuring comprehensive national coverage and minimizing selection bias. The sampling approach was intended to produce a representative subset of the national dairy herd population, thereby allowing for general estimates of apparent antibody prevalence and robust evaluation of the ELISA test under field conditions.

Herds included in the sample were geographically distributed across all major dairy-producing regions, reflecting the overall structure of the dairy sector in the country. Bulk tank milk samples were collected through the routine milk delivery system, facilitated by national legislation requiring milk processors to collect and submit samples to the Eurofins from all milk supplying herds. This infrastructure enabled efficient and uniform sample collection from 100% of the selected herds.

Results & Discussion

ELISA and PCR both offer complementary strengths in the surveillance of Mycoplasma bovis in bulk tank milk (BTM). While ELISA is effective for detecting herd-level exposure over time, PCR enables rapid identification of active infections. The choice of method should reflect specific surveillance goals—whether the aim is early detection of shedding animals or estimation of past or present infection prevalence at the herd level.

In this study, 402 BTM samples were analyzed using both ELISA and PCR. Only four samples (1%) tested positive by PCR, and all four were also antibody-positive by ELISA. In contrast, ELISA revealed a much higher level of exposure, with an average antibody positivity rate of 46% across all BTM samples. Notably, there was no clear relationship between PCR and ELISA results, indicating that antibody detection reflects historical or subclinical infections rather than ongoing bacterial shedding. This highlights the



importance of interpreting ELISA results in the context of infection dynamics and the limitations of PCR in detecting intermittent shedding.

The use of both PCR and ELISA in parallel can improve diagnostic accuracy and support more informed herd-level control strategies. Factors such as cost, testing frequency, and the expected stage of infection within the herd should guide the selection of diagnostic tools.

Figure 1 presents the regional distribution of antibody prevalence across the sampled dairy herds, illustrating variation in exposure levels between different parts of Denmark.

Figure 1: Distribution of sampling and result

Conclusion

The significant discrepancy between ELISA and PCR results highlights the importance of understanding what each method detects—exposure versus active infection. ELISA identified a higher number of positive herds, suggesting broader historical exposure, while PCR detected only a subset with active shedding. This gap emphasizes that relying on a single diagnostic method may lead to underestimation of herd infection status. A combined testing strategy is recommended to obtain a more complete epidemiological picture and guide effective control measures.

References

Marquetoux, N., Vignes, M., Burroughs, A., Sumner, E., Sawford, K., & Jones, G. (2023). Evaluation of the accuracy of the IDvet serological test for Mycoplasma bovis infection in cattle using latent class analysis of paired serum ELISA and quantitative real-time PCR on tonsillar swabs sampled at slaughter. PLOS ONE, 18(5), e0285598. https://doi.org/10.1371/journal.pone.0285598

Nobrega, D. B., French, J. E., & Kelton, D. F. (2023). A scoping review of the testing of bulk milk to detect infectious diseases of dairy cattle: Diseases caused by bacteria. Journal of Dairy Science, 106(3), 1986–2006. https://doi.org/10.3168/jds.2022-22395

Salgadu, A., Burroughs, A., Sawford, K., Johnstone, T., Wawegama, N. K., Stevenson, M. A., Browning, G. F., & Firestone, S. M. (2025). Cut-off evaluation of ID Screen Mycoplasma bovis ELISA for use on bulk tank milk in New Zealand. Preventive Veterinary Medicine, 240, 106528. https://doi.org/10.1016/j.prevetmed.2025.106528

Potential of sensor systems monitoring animal health to support treatment decisions in clinical mastitis

Karin Knappstein[1*], Tim Roggensack[1], Kerstin Barth[2] and Alexandra Beckmann[1]

[1] Institute of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany

[2] Institute of Organic Farming, Johann Heinrich von Thünen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, Trenthorst, Germany

***Corresponding author's email: karin.knappstein@mri.bund.de**

Introduction

With regard to udder health, sensor systems for animal health are expected to early detect health disturbances and generate alerts indicating suspected mastitis (Hogeveen et al., 2010). The challenge is to achieve high sensitivity and specificity to limit the number of diseases remaining undetected but also the number of false positive alarms. Reduction in rumination time of individual cows has been observed in different illnesses (Beauchemin, 2018). In addition to generation of automatic alerts, data may be used to improve the evaluation of the actual status of an already detected disease. In the context of clinical mastitis, rumination patterns may be interpreted as additional information on severity of disease and to support treatment decisions by estimating the causative pathogen e.g. based on sudden onset.

During an outbreak of mastitis caused by *Klebsiella pneumoniae*, sensor data were evaluated and compared to data from clinical mastitis cases caused by *Streptococcus uberis* during the same time period.

Material & Methods

All lactating cows of the Research Farm Schaedtbek of the Max Rubner-Institute (Kiel, Germany) were equipped with neck-bend sensors monitoring activity and rumination. Naturally occurring clinical cases of mastitis during September to November 2024 were evaluated regarding severity of disease according to the following symptoms (IDF, 2022): mild (1) = abnormal milk, moderate (2) = inflammatory signs of the infected quarter, severe (3) = general symptoms of systemic illness (fever, anorexie). On detection of clinical mastitis during milking, quarter milk samples were immediately taken by farm personnel for bacteriological analysis according to the guidelines of the German

Veterinary Medical Society (2018). Rectal temperature was measured manually. Sensor data on activity per cow and hour were provided by MSD Animal Health (Munich, Germany) and summed up to 12 h-periods for evaluation. In addition, automated alerts were included based on a health index as a combination of activity and rumination or lacking rumination over a 12 h-period. Cows with recurring mastitis or mixed infections were excluded from the evaluation.

Results & Discussion

Independent of severity of disease cows with clinical mastitis caused by *Streptococcus uberis* showed only a mild reduction of rumination activity and no complete cessation of rumination within the 12 h-intervals (Figure 1).

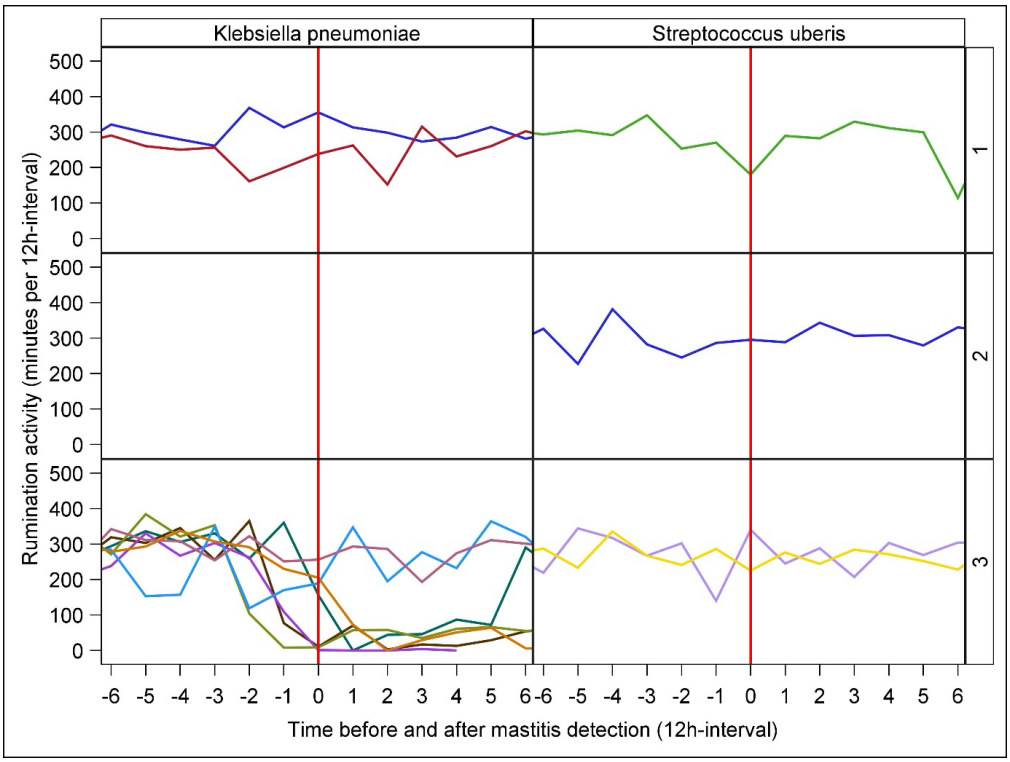


Figure 1: Rumination activity (minutes per 12 h-interval) in cows with clinical mastitis caused by *Klebsiella pneumoniae* (n = 9; left side) or *Streptococcus uberis* (n = 4; right side) according to severity of disease (1 = mild, 2 = moderate, 3 = severe) and in relation to detection of disease (0 h, red vertical line)

From seven cows with severe *Klebsiella* mastitis, five cows showed complete cessation of rumination activity in the course of the disease within the time period of 12 h before detection by farm personnel until 24 h after detection of clinical symptoms (Figure 1). Automated alerts were produced for seven cows with *Klebsiella* infection within a time period of 15 hours before to 36 hours after detection of clinical mastitis. Two cows were euthanized 2 and 14 days after onset of disease, respectively. Only for one of the four cows with *Streptococcus uberis* mastitis an alert was sent 48 h after detection of symptoms. According to Oliveira et al. (2013) 75 % of severe cases of mastitis were caused by gram-negative pathogens and consequences regarding survival of cows and milk yield reduction were more serious in mastitis caused by *Klebsiella* spp. than by

Escherichia (E.) coli. Using a combination of rumination and activity, Stangaferro et al. (2016) detected mastitis caused by *E. coli* with higher sensitivity than mastitis caused by other pathogens, including *Klebsiella* spp. This is in accordance with different severity scores caused by gram-negative bacteria (Oliveira et al., 2013) and our observations on different courses of rumination even in severe cases of *Klebsiella* mastitis.

Although there is already consensus that antimicrobial therapy of severe cases of clinical mastitis should not be delayed until culture results are available (de Jong et al., 2023), the selection of the appropriate antimicrobial may be supported by estimating the causative organism. The observed sudden decrease in rumination appears to be more probable for gram-negative mastitis pathogens and if observed, antimicrobials targeting these bacteria should be selected for prompt therapy without delay.

Conclusion

Prudent use of antimicrobials implies the targeted application with regard to the pathogen involved. According to our observations, sensor data may provide a valuable contribution for directing treatment decisions at first occurrence of disease or during the course of the disease. It remains to be evaluated with a greater dataset, if the observations can be confirmed and if sensor data provide an added value compared to the already available data from modern milking systems.

References

Beauchemin, K.A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science*, *101*(6), 4762-4784.

De Jong, E., McCubbin, K.D., Speksnijder, D., Dufour, S., Middleton, J.R., Ruegg, P.L., Lam, T.J.G.M., Kelton, D.F., McDougall, S., Godden, S.M., Lago, A., Rajala-Schultz, P.J., Orsel, K., De Vliegher, S., Krömker, V., Nobrega, D.B., Kastelic, J.P. & Barkema, H.W. (2023). Invited review: Selective treatment of clinical mastitis in dairy cattle. *Journal of Dairy Science*, *106*(6), 3761-3778.

German Veterinary Medical Society. (2018). [Guidelines for antiseptic milk sampling and guidelines to isolate and identify mastitis pathogens]. 3rd ed. Verlag der Deutschen Veterinärmedizinischen Gesellschaft e.V., Gießen.

Hogeveen, H., Kamphuis, C., Steeneveld, W. & Mollenhorst, H. (2010). Sensors and clinical mastitis - the quest for the perfect alert. *Sensors*, *10*(9), 7991-8009.

International Dairy Federation (IDF). (2022). Guidelines for defining quarter and udder health status and cured clinical and subclinical mastitis cases. *Bulletin of the IDF,* No. 515/2022.

Oliveira, L., Hulland, C. & Ruegg, P. L. (2013). Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. *Journal of Dairy Science*, *96*(12), 7538–7549.

Stangaferro, M.L., Wijma, R., Caixeta, L.S., Al-Abri, M.A., Giordano, J.O. (2016). Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. *Journal of Dairy Science*, *99*(9), 7411-7421.

Perceptions Drive Decisions: How Farmers Understanding and Use of Technology Shape Mastitis Management in Automatic Milking Systems

Dorota Anglart[1], Lisa Ekman[2][3]*, Nina Lind[4], Gabriela Olmos Antillón[2]

[1]DeLaval International AB, Tumba, Sweden

[2]Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

[3]Växa Sverige, Stockholm, Sweden

[4]Department of Economics, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

***Corresponding author: dorota.anglart@delaval.com**

Thematic area: Udder Health

Introduction

Mastitis remains the most costly and welfare-critical disease in dairy cows. With the widespread use of automatic milking systems (AMS), farmers' roles in udder-health management have shifted from visual and physical direct observation of the cows to the interpretation of digital information. This transformation changes how farmers perceive health, risk, and control and consequently how they act when a cow develops mastitis.

While traditional models of mastitis control have emphasised biological and economic factors, recent research shows that farmers' perceptions of their herd's health status, their own control, and the meaning of animal welfare are decisive for their behaviour. These perceptions are shaped by technology design, farm context, and personal motivation. Understanding this perception–decision dynamic is essential for designing advisory and technological systems that support effective and sustainable udder-health management.

This work integrates large-scale survey and qualitative data from Swedish dairy farms to

explore how farmers construct, interpret, and act upon udder-health information provided by AMS.

Material & Methods

A national survey of Swedish dairy farms using AMS (n = 246) explored farmer's decision making in relation to available technology on the farm, herd-level udder health, indicated by bulk milk somatic cell count (SCC), and other farm and farmer characteristics. Three realistic mastitis scenarios captured how farmers would detect a cow with an udder health problem, as well as at which point a farmer would contact a veterinarian or initiate treatment, serving as an indicator of health-seeking behaviour (Ekman et al. 2025a).

Psychological constructs such as perceived herd udder health, perceived control serving as constructs measuring farmers illness perception (Leventhal et al., 2001), and motivational values (economic and non-economic; Hansson et el. 2016), were assessed through validated Likert-scale items. Statistical modelling using Serial Mediation Analyses (Hayes et al. 2017) identified pathways linking actual herd SCC to behavioural intentions through these perceptions and motivations.

To complement the quantitative analysis, in-depth interviews with Swedish AMS users (n = 9) provided qualitative insight into how farmers interpret and use AMS data for mastitis detection. A reflexive thematic approach examined meanings attached to technology, data, and animal observation. Integration of quantitative and qualitative findings followed a critical realism framework, linking objective patterns with underlying mechanisms (Ekman et al. 2025 b).

Results & Discussion

Perceptions and Motivation as Drivers of Action

Measured udder-health status (average herd SCC) showed no direct link to how actively farmers sought veterinary help. Instead, subjective perception was central (Ekman et al. 2025a). Farmers who viewed their herd's udder health as good and believed they had strong control over it were more proactive in responding to mastitis cases. Motivational orientation further differentiated behaviours. Farmers guided by noneconomic values, such as preventing suffering or maintaining pride in healthy animals, and by economic motives, having a profitable production, acted more promptly. These findings demonstrate the complexity of mastitis management.

How Perceptions Are Constructed

Farmers' perceptions are continuously shaped by their interaction with cows and technology. Despite AMS generating large volumes of health data, most farmers relied on behavioural data (e.g., cows late to milking) as the first sign of an issue. The automated alerts were more often used for confirmation rather than detection. Trust in AMS information varied with sensor features. Farmers using systems with built-in SCC sensors expressed a high confidence in that specific sensor for automated detection, whereas farmers without SCC sensors relied on other types of information, often from

several combined sources. Several farmers described data overload and difficulties in adapting and interpreting the data according to their needs. Across farms, the decision to act on mastitis information emerged from a negotiation between human judgement and technological signals. Farmers integrated data through their own practical reasoning, balancing system alerts with intuition, knowledge of individual cows, and farm routines. This synthesis process is where perceptions are built: the AMS does not replace the farmer's eye, the "djuröga", but it reshapes what the farmer sees. Thus, the technical environment influences how information becomes meaningful and actionable and it is crucial to support farmers in interpreting and trusting AMS data, without losing the experiential dimension of care.

Integrative Interpretation

Combining behavioural-psychology and technology-adoption perspectives reveals a coherent perception-to-decision pathway:

Objective signals (e.g., SCC, AMS alerts) → Subjective perception of herd health and control → Motivational evaluation (economic + noneconomic) → Decision to act (e.g., call vet, treat, monitor) (Ekman et al.2025b).

Perceptions serve as the cognitive filter through which data acquire meaning. Consequently, advisory efforts or system designs that neglect the farmer's own perception of herd health may not be effective. Training programs that frame AMS data within farmers' existing sense of control and animal welfare values may achieve greater behavioural change than purely technical instruction.

Conclusion

The relationship between farmers' perceptions, technology and motivation shapes their decision making and herd health strategies. Efforts to improve udder-health in farms with AMS should therefore acknowledge perception and motivation as key behavioural determinants of management actions. There is a need to enhance farmers' interpretive skills through participatory advisory programs; and the co-design AMS interfaces that reinforce farmers' sense of control and understanding.

References

Ekman, L., Fall, N., Emanuelson, U., & Lind, N. (2025a). Farmer attitudes and motivation affect their health-seeking behaviour in relation to mastitis in dairy cows. J. Dairy Sci.

Ekman, L., Anglart, D., Gillsjö, I., Lind, N., Fall, N., & Olmos Antillón, G. (2025b). What information counts when detecting mastitis in automatic milking systems? —A mixed methods approach from a Swedish perspective. J. Dairy Sci.

Hansson, H., & Lagerkvist, C. J. (2016). Animal Welfare, 25(4), 459–467.

Hayes, A. F., & Rockwood, N. J. (2017). Behavior Research Methods, 49, 1–21.

Leventhal, H., et al. (2001). Psychology & Health, 16(1), 1–20.

Effective pre-milking hygiene protocols will contribute to reduce contamination of the milking equipment, milk and cows

Sofie Piepers[1,2], Kristien Mertens[1] and Adrien Tavel[3]

[1] University of Ghent, Merelbeke, Belgium

[2] MEXCELLENCE BV, Sint-Denijs-Westrem, Belgium

[2] CID LINES, an ECOLAB company, Ieper, Belgium

***Corresponding author's email: Sofie.Piepers@UGent.be**

Introduction

Effective udder hygiene is essential for maintaining milk quality and preventing intramammary infections (IMI) in dairy cows. One of the most critical steps in the pre-milking routine is the thorough cleaning and disinfection of teats using techniques such as pre-dipping or pre-foaming. This practice aims to remove visible dirt, organic matter, and transient microorganisms from the teat surface before milking, thereby reducing the risk of bacteria entering the teat canal and causing new udder infections (Galton et al., 1988).

Lactic acid, a naturally occurring organic acid, has gained attention as a biodegradable and environmentally safe disinfectant with strong antimicrobial activity against the major mastitis pathogens (Damasceno et al., 2025). Studies investigating the use of lactic acid as a pre-milking foam have primarily been conducted under pasture-based milking systems, where environmental exposure and contamination risks differ from those in confined or intensive housing systems (Fitzpatrick et al., 2019). Consequently, there remains limited understanding of its performance and effects in pre-foaming formulations under alternative management conditions, particularly in high-yield, housed dairy herds.

The present study aims to investigate the effects of lactic acid-based pre-foaming (Kenopure Pro™) on the colonization of teat skin in dairy cows, employing both *in vitro* and *in vivo* methods. By characterizing its potential impact on teat skin microbiota, this work seeks to contribute to the development of optimized, sustainable pre-milking

protocol that balance bacterial control, skin health, and animal welfare across diverse production systems.

Material & methods

The set-up of the *in vitro* study was adapted from Piepers et al. (2020). Sixteen 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 109 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 & Discussion

The main findings of the *in vitro* study are summarized in Table 1. In the in vitro experiment, all negative control swabs from rubber calf nipples remained culture-negative, regardless of the teat preparation method. Wiping with a clean and dry paper towel substantially reduced bacterial counts compared to no cleaning. However, pre-foaming with lactic acid, followed by either immediate wiping or wiping after a 30-second contact time, achieved a greater reduction in bacterial load, demonstrating superior efficacy compared to dry wiping alone.

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™. [5]Swab of outside of rubber calf nipple. [6]Serial dilution of 1 mL of immersion solution in which rubber calf nipple was soaked for 30 min.

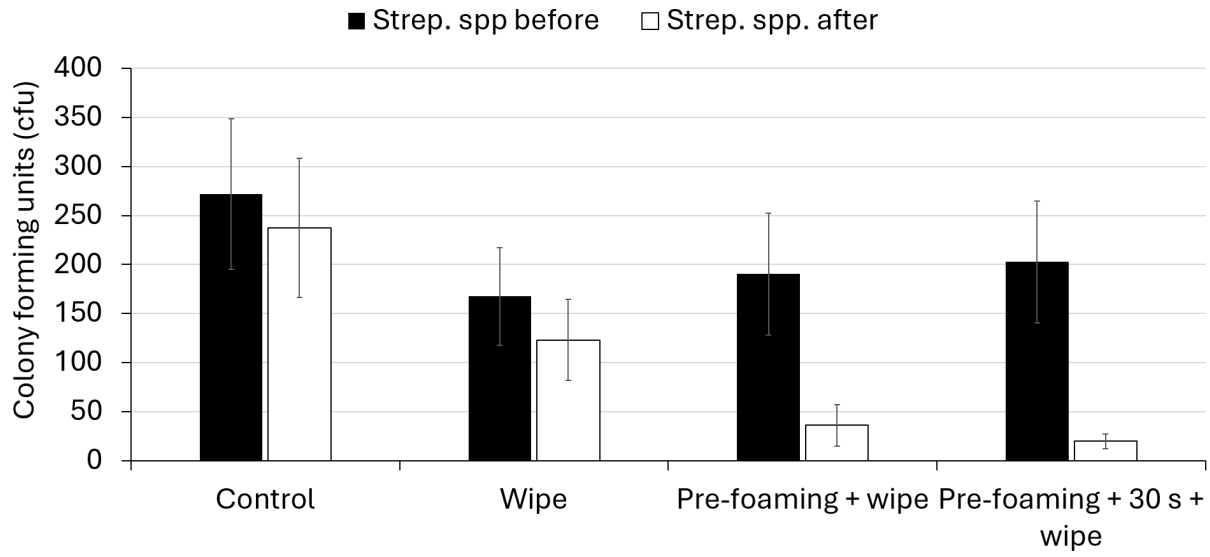


Figure 1. Average colony forming units of *Streptococcus* spp. (+/- standard error of the mean) before and after teat preparation in the different teat preparation groups.

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.

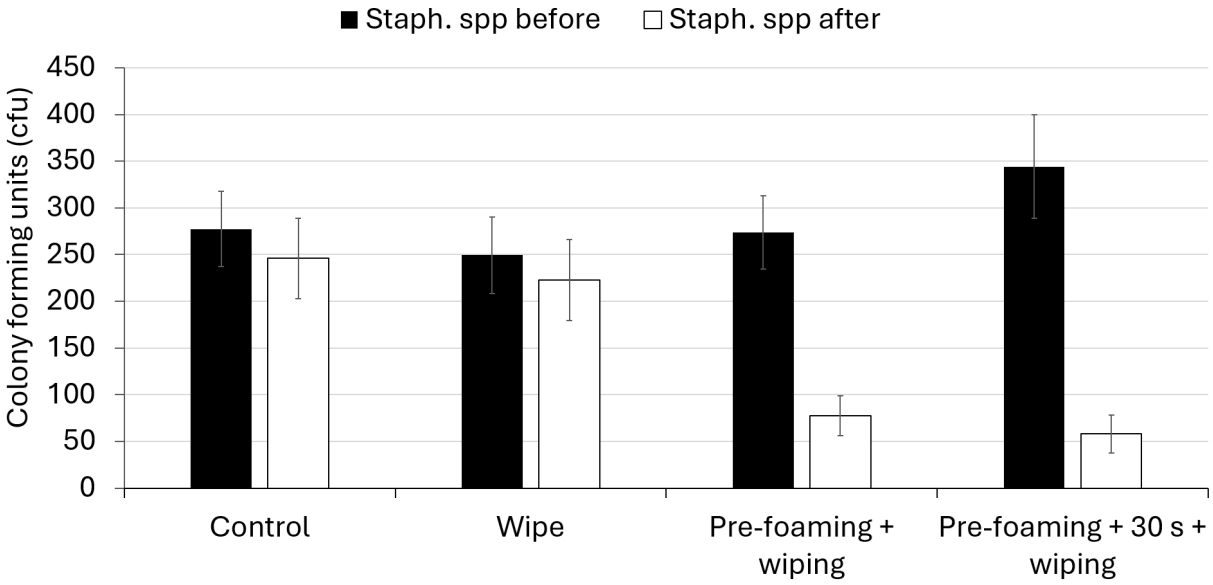


Figure 2. Average colony forming units of *Staphylococcus* spp. (+/- standard error of the mean) before and after teat preparation in the different teat preparation groups.

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 lactatic acid (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.

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 lactic acid (Kenopure Pro™) proved more effective, regardless of the contact time. Consistent with other findings (1), teat skin colonisation *in vivo* 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.

References

Damasceno, M. D., Gonçalves, M. S., Pinto da Silva, B. H., Carneiro, G. B., Reis Gonçalves, A., Reis Pereira, B., Costa Borges Reis Trolesi, A. C., de Sousa Bueno Filho, J. S., Domeles Seles, E. M., & de Sá Guimarães, A. 2025. Susceptibility of mastitis-causing pathogens (Escherichia coli and Staphylococcus aureus) to disinfectants used as teat dipping. *Veterinary Microbiology*, 309, 110678. DOI: 10.1016/j.vetmic.2025.110678

Fitzpatrick, S. R., Garvey M., Flynn, J., O'Brien B. & Gleeson D. (2021). Effect of pre-milking teat foam disinfection on the prevention of new mastitis rates in early lactation. *Animals, 11*, 2582-2596. DOI: 10.3390/ani11092582

Galton, D. M, Peterson, L. G., & Merrill, W. G. (1988). Evaluation of udder preparations on intramammary infections. *Journal of Dairy Science, 71*, 1417-1421. DOI: 10.3168/jds.S0022-0302(88)79700-3

Piepers, S., Van Den Brulle, I., Mertens K., & De Vliegher, S. 2020. Short communication: Barrier characteristics of 3 external teat sealants to prevent bacterial penetration under in vitro conditions using rubber calf-feeding nipples. *Journal of Dairy Science*, *103*, 6569-6575. doi: 10.3168/jds.2019-17575

Omitting treatment of minor pathogens in quarter-selective dry cow therapy: Antibiotic consumption and cure rates

Alexandra Beckmann [1*], Kerstin Barth[2] and Karin Knappstein[1]

[1] Institute of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany

[2] Institute of Organic Farming, Johann Heinrich von Thünen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, Trenthorst, Germany

***Corresponding author's email: alexandra.beckmann@mri.bund.de**

Introduction

Due to concerns about antimicrobial resistance, a targeted and prudent use of antibiotics becomes essential in animal production. The highest use of antibiotic therapy in dairy farming is for treating mastitis, particularly at dry-off (Kuipers et al., 2016). Significant reductions in antibiotic use can be achieved at dry-off when treatment is reserved for individual quarters. The introduction of internal teat sealants (ITS) as effective, non-antibiotic alternative (Pearce et al., 2023) and the fact that many cows have only one or two infected quarters at dry-off (McDougall et al., 2021) have renewed the focus on individual quarter treatment. Various quarter-level attempts based on rapid culture (Kabera et al., 2020; Rowe et al., 2020) or California Mastitis Test results (McDougall et al., 2022) have shown that quarter-selective dry cow therapy (QSDCT) is possible with a substantial reduction of antibiotic use, but less is known about limiting antibiotic treatment solely based on the detected pathogen. Therefore, we investigated a QSDCT approach in commercial dairy farms with treatment of major pathogens and non-treatment of minor pathogens at dry-off.

Material & methods

The study was conducted between February 2021 and December 2022 in 16 German dairy farms with herd sizes ranging from 80 to 1,280 cows (Beckmann et al., 2025a). The majority of mastitis pathogens in these herds were of environmental origin (Beckmann

et al., 2025b). Treatment with antibiotics at dry-off was strictly based on the results of bacteriological analysis performed on quarter milk samples collected two weeks prior to drying off. All samples were collected by farm personnel and analyzed according to the guidelines of the German Veterinary Medical Society (2018). Only quarters infected with major bacterial pathogens were treated with antibiotics. To prevent new intramammary infections, all quarters received an ITS. A second sample was taken three to five days after calving to determine cure rate and new intramammary infection rate.

Results & Discussion

Antibiotic consumption

A total of 4,530 quarters from 1,155 dry periods were included in the analysis. Although all farms had previously used selective dry-off strategies on cow level, the antibiotic use was further reduced to 8.1% of all quarters (varying from 2.6% to 28.8% on individual farms). Compared to McDougall et al. (2022) and Kabera et al. (2020), antibiotic use was eight and about five times lower in this study. If consistent treatment of minor pathogens had been part of the strategy, antibiotic use would have been 2.6 times higher.

Bacteriological cure rate

A cure rate of 97.1% was determined for udder quarters treated with antibiotics (Table 1). These high cure rates are consistent with findings from other quarter-level approaches (Kabera et al., 2020; McDougall et al., 2022). Depending on the mastitis pathogens, the cure rates varied from 86.2% (*Staphylococcus aureus*) to 100.0% (*Streptococcus dysgalactiae*). Minor pathogens (Non-*aureus* staphylococci and *Corynebacterium* spp.) were not treated with antimicrobials and self-cure rates of more than 80% were observed (Table 1).

Table 1. Bacteriological cure rates of infections caused by major and minor pathogens at dry-off

	Quarters	**Cured[1] quarters**	**Bacteriological cure rate**	
			Total	**Pathogen-specific**
Microbiological findings	**(n)**	**(n)**	**(%)**	**Min – Max (%)**
Major pathogens[2] (with antibiotics)	312	303	97.1	86.2 – 100.0
Minor pathogens[3] (without antibiotics)	577	474	82.1	81.6 – 83.0

[1] including changing of the detected pathogen before and after drying off.

[2] *S. aureus, Str. dysgalactiae*, *Str. uberis*, other esculin-positive streptococci, enterococci, Gram-negative bacteria (including *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Proteus* spp., *Pasteurella spp. and Pseudomonas spp.*), *Trueperella pyogenes*, Mixed infection.

[3] Non-*aureus* staphylococci and *Corynebacterium* spp.

Challenges in implementation

It is important to consider that QSDCT based on bacteriological outcomes is accompanied by some challenges for dairy farmers, such as the antiseptic sampling, the organizational effort, or the correct quarter allocation. Due to increase of time for sampling and cost of bacteriological analysis, further analyses on the preselection of cows for sampling with infections caused by major pathogens are needed.

Conclusion

QSDCT based on microbiological analysis of quarter milk samples can be successfully implemented on commercial dairy farms. High self-cure rates of untreated minor pathogens indicate no need for use of antibiotics at dry-off. The pathogen-based QSDCT approach does reduce antibiotic consumption and also leads to a more targeted use of antibiotics on quarters with infections caused by major pathogens.

References

Beckmann, A., Barth, K., & Knappstein, K. (2025a). Investigation of quarter-selective dry cow therapy based on bacteriological outcomes on dairy farms. *Journal of Dairy Research*, *accepted*.

Beckmann, A., Barth, K., & Knappstein, K. (2025b). Comparison of two sampling approaches to determine the prevalence of mastitis pathogens in dairy herds. *Journal of Dairy Research*.

German Veterinary Medical Society (2018) [Guidelines for Antiseptic Milk Sampling and Guidelines to Isolate and Identify Mastitis Pathogens]. 3rd ed. Verlag der Deutschen Veterinärmedizinischen Gesellschaft e.V., Gießen.

Kabera, F., Dufour, S., Keefe, G., Cameron, M., & Roy, J.-P. (2020). Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. *Journal of Dairy Science*, *103*(8), 7276–7287.

Kuipers, A., Koops, W. J., & Wemmenhove, H. (2016). Antibiotic use in dairy herds in the Netherlands from 2005 to 2012. *Journal of Dairy Science*, *99*(2), 1632–1648.

McDougall, S., Williamson, J., Gohary, K., & Lacy-Hulbert, J. (2021). Detecting intramammary infection at the end of lactation in dairy cows. *Journal of Dairy Science*, *104*(9), 10232–10249.

McDougall, S., Williamson, J., & Lacy-Hulbert, J. (2022). Bacteriological outcomes following random allocation to quarter-level selection based on California Mastitis Test score or cow-level allocation based on somatic cell count for dry cow therapy. *Journal of Dairy Science*, *105*(3), 2453–2472.

Pearce, S. D., Parmley, E. J., Winder, C. B., Sargeant, J. M., Prashad, M., Ringelberg, M., Felker, M., & Kelton, D. F. (2023). Evaluating the efficacy of internal teat sealants at dry-off for the prevention of new intra-mammary infections during the dry-period or clinical mastitis during early lactation in dairy cows: A systematic review update and sequential meta-analysis. *Preventive Veterinary Medicine*, *212*, 105841. https://doi.org/10.1016/j.prevetmed.2023.105841

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J., & Thomas, M. J. (2020). Randomized controlled non-inferiority trial investigating the effect of 2 selective dry-cow therapy protocols on antibiotic use at dry-off and dry period intramammary infection dynamics. *Journal of Dairy Science*, *103*(7), 6473–6492. https://doi.org/10.3168/jds.2019-17728

Frequent online cell count measurements enables and motivates farmers and advisors to work proactively with udder health

John M. Christensen [1], Karin Ulvshammar[2], Ditsa Panchal[2] and Ilka Klaas[2*]

[1] Lattec I/S, Hillerod, Denmark

[2] DeLaval International AB, Tumba, Sweden

***Corresponding author's email: ilka.klaas@delaval.com**

Introduction

In automatic milking systems (AMS), farmers need to consistently monitor udder health for timely detection of cows with mastitis and the development of udder health key parameter indicators (KPIs) over time. Typically, mastitis detection in AMS is based on deviations in electrical conductivity and other milking behaviours presented to the farmer on a user interface or in an alert lists. With addition of specific sensor systems measuring somatic cell counts (SCC), farmers are enabled to use this key parameter to monitor udder health on an individual cow and herd level. A previous on-line SCC sensor system (DeLaval online cell counter OCC) has shown high performance (Fadul-Pacheco et al. 2018, Nørstebø et al. 2019). A recent Swedish study showed that farmers with online SCC sensor systems use online SCC information intensively and in combination with the AMS data (Ekman et al. 2025). DeLaval recently launched the next generation of an on-line SCC sensor system that has an advanced biomodel, more sustainable consumables, and provides improved accuracy of measurements. This novel system, DeLaval BioSensors milk cell analysis MCA, offers both mastitis detection and herd management tools. For optimal use in udder health management, high performance of the system is important. Our objective was to validate the new MCA cell count instrument against a gold standard and illustrate a method to determine the correlation between the measurements.

Material & methods

The MCA is connected to a separate device, the DeLaval milk sample unit MSU, which is transporting the milk sample from the end unit in VMS V300, via the milk outtake valve, to the MCA. The MSU contains a cleaning device, which is cleaning itself and the

connected sensors, every 6 hours, to meet cleaning standards, which is a requirement to meet a high sampling performance. The MSU contains a milk collector with an air gap, that prevents milk from going backwards to the cooling tank, which is a requirement for food compliance (FDA standard) in the USA.

The MCA validation test was carried out in 2 Swedish dairy herds with 3 AMS units, each equipped with an MCA sensor system. The MCA validation was carried out on two farms with three AMS units each farm, and each equipped with an MCA sensor system. The validation period was from week 24, 2024 to week 11, 2025. Once weekly a validation test was carried out.

During milking the milk from all four quarters is collected in the AMS milk receiver. When the milking of a cows is close to the end, an agitator is activated to make sure the milk is homogeneous when it is pumping to the cooling tank. In the pump house there is an outlet for the MSU and the milk recording device (MRD) to automatically take out two fractions of the same milk. The MSU fraction is pushed to the MCA. The MRD fraction is stored in tubes with Bromol for preservation of the milk until it is in the external laboratory. In the MCA is the milk mixed with stainer for coloring the milk cells. A camera takes an image and SCC is counted. The MRD fraction was analyzed in an external laboratory (EL) accredited according to the European standard EN ISO/IEC 17025:2017.

Data from the Delpro herd management program consisting of cow ID, date, and MCA SCC result was exported in Microsoft Excel and matched with the results reported from Eurofins laboratory for the same cow and date. Differences between the MCA SCC and the laboratory SCC were calculated as the results given from the AMS minus the laboratory results.

The results from the MCA were compared to the EL to prove the MCA accuracy. The scientific method for comparing on farm SCC and EL SCC was applied using the statistical equation (Lin, 1989) for Concordance Correlation Coefficient (CCC).

Results & Discussion

During the study period, a total of 7131 MCA SCC samples was taken in the two study herds and successfully matched with SCC reference laboratory test results, after discarding 1901 samples where cow ID could not be matched or MCA or the lab test sample was not taken correctly.

CCC was 0.92, thus exceeding the ICAR recommended threshold of 0.89 and similar to the CCC of 0.91 for the previous DeLaval OCC reported by Fadul Pachero et al. (2018).

Figure 1 displays the correlation between MCA SCC and external reference laboratory SCC used as gold standard, all samples.

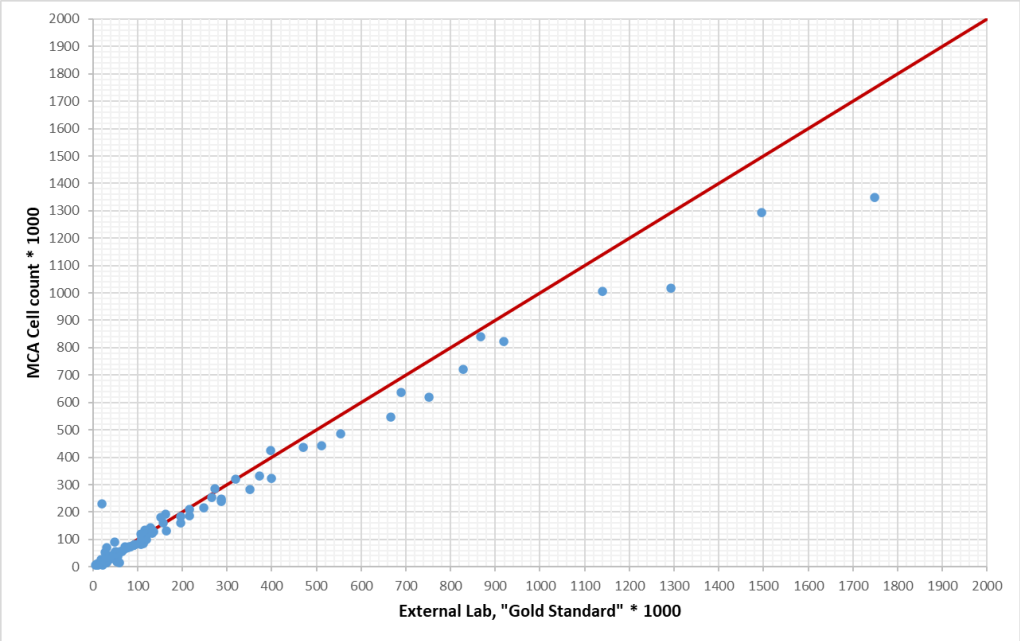


Figure 1. On-line MCA Cell counts (MCA SCC) plotted against Somatic Cell Count (SCC, 1000/ml) measured in a DHI reference laboratory from two dairy herds at weekly samplings (n = 7131 observations). The superimposed 45 ° line represents perfect agreement between the two methods.

While the overall CCC between MCA SCC and laboratory SCC was high, figure 1 suggests that the agreement is higher when SCC is lower due to slightly lower values measured by the MCA. Despite this underestimation at high SCC, ICAR recommendation can be achieved. From a biological cow health perspective, cows exceeding 1 million cells/ml are certainly affected by mastitis and thus will be detected by the MCA. Typically, 200000 cells/ml are often used as threshold for subclinical mastitis. As the results show high accuracy especially at lower levels, MCA based KPIs on subclinical mastitis enable farmers to early detect subclinical mastitis and to monitor recovery, chronicity and eventually relapses. Farmers are enabled to use the provided information for detailed treatment and prevention strategies during lactation and at dry-off. The targeted use of frequent on-farm SCC has the potential to reduce use of antibiotics, improve milk quality and identify cows and groups at risk of mastitis.

Conclusion

This first validation study on the online SCC sensor system DeLaval MCA shows that accurate information is provided for farmers, enabling intensive monitoring of udder health and milk quality. Animals in need of farmer and veterinary attention can be detected early and group level SCC data used to identify trends in the herd. More studies are needed to confirm the performance in different herd settings and explore best herd strategies.

References

Ekman,L., Anglart, D., Gillsjö, I., Lind, N., Fall,N., & Olmos Antillón, G. (2025). What information counts when detecting mastitis in automatic milking systems? A mixed

methods approach from a Swedish perspective. Journal of Dairy Science 108, 9861–9875. https://doi.org/10.3168/jds.2025-26455

Fadul-Pacheco, L, Lacroix, R, Séguin, M., Grisé, M., Vasseur, E., & Lefebvre, D.M. (2018). Characterization of milk composition and somatic cell count estimates from automatic milking systems sensors. Proceedings ICAR Conference 2018, Auckland, New Zealand, 53-63.

Nørstebø, H, Dalen, G., Rachah, A., Heringstad, B., Whist, A. C., Nødtvedt, A. & Reksen, O. (2019). Factors associated with milking-to-milking variability in somatic cell counts from healthy cows in an automatic milking system. Preventive Veterinary Medicine 172 (2019) 104786. https://doi.org/10.1016/j.prevetmed.2019.104786

Addressing antimicrobial resistance: A case study on the receptivity of Australian dairy farmers to a novel alternative treatment for bovine mastitis

Tiarna Scerri [1] and Jennifer Manyweathers[2*]

[1]Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW 2650, Australia

[2]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga 2650, Australia

[*]Corresponding contact: tscerri@csu.edu.au

Introduction

The increasing prevalence of antimicrobial resistance genes within common mastitis-causing bacterial strains (Morales-Ubaldo et al., 2023; Naranjo-Lucena & Slowey, 2023; Molineri et al., 2021) reinforces the need for research into alternatives to conventional antimicrobial treatments.

Such alternatives should not only be effective and sustainable, but sufficiently practical for dairy farmers to implement on-farm. However, there is minimal understanding of what dairy farmers believe to be a palatable treatment that they would be receptive to implementing. This gap in knowledge crucially limits the capacity of current researchers to establish and prioritise research directions that will be supported by dairy farming communities.

Material & Methods

We conducted a social investigation that aimed to capture the perspectives of Australian dairy farmers on antimicrobial resistance in the context of mastitis, as well as their receptivity towards novel, omic-based alternative treatments and general preferences for treatment format, mode of action, delivery and on-farm implementation. An in-depth, online survey – hosted by Qualtrics® – was disseminated through various personal and professional channels. This survey collected data from consenting participants about their dairy farm, mastitis management protocols and views and preferences on emerging alternative treatments. A mixed-methods analysis approach

(Morse & Niehaus, 2009) was implemented on a final dataset sample size of 20 individuals. This approach involved traditional descriptive analyses, iterative thematic analysis (Morgan & Nica, 2020) and Bayesian Network modelling (Kjærulff & Madsen, 2008; Korb & Nicholson, 2011).

Results & Discussion

The resulting, multi-faceted dataset was rich and diverse in its findings. In addition to findings based on individual data variables, an especially poignant aspect of our results was the finalisation of a Bayesian Network (Figure 1). This probabilistic model illustrated many of the collected data variables and allowed the examination of their interdependent relationships with each other and a subjectively assessed level of farmer 'receptivity'.

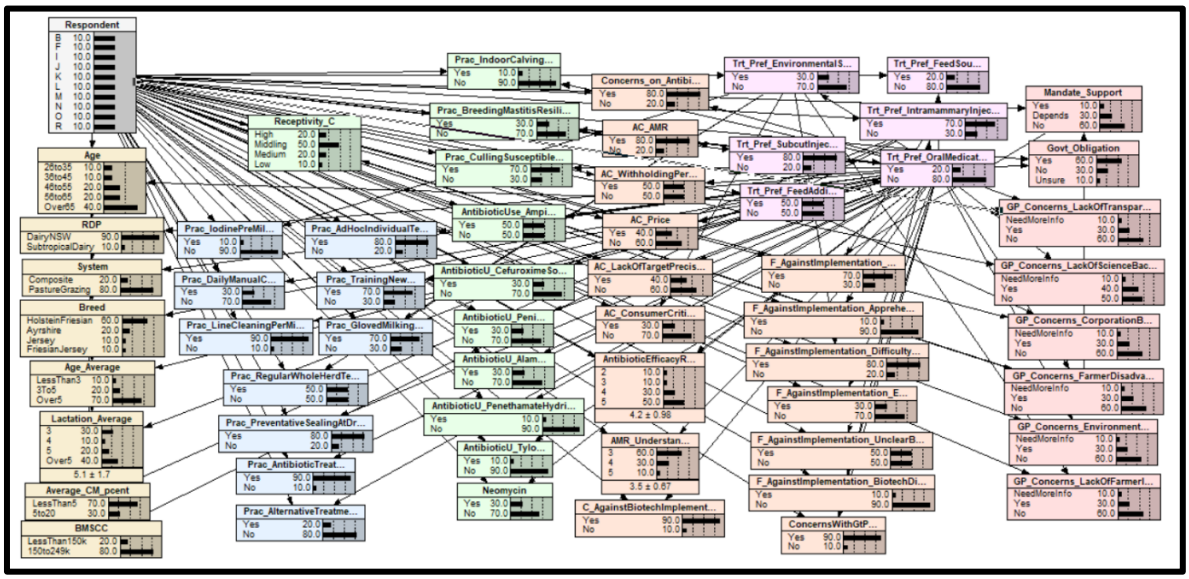


Figure 1. Bayesian Network model generated with Netica®. This model is interactive, and so the depth of its utility cannot be accurately shown through a Word file.

Overall, the key insights are as follows: Quantitative and qualitative data points reinforced that the respondents collectively displayed a general receptivity towards the concept of a novel, omic-based alternative treatment for mastitis. Sensitivity analyses from the Bayesian Network revealed that such farmer receptivity is most influenced by age and their rating of current antibiotic efficacy. Further thematic analysis identified that the barrier towards stronger receptivity is caution against processor and consumer ramifications. These findings importantly establish general support for alternative treatments and identify barriers to be addressed before the attempted implementation of any novel treatment.

Dual analysis of preference scale and qualitative data showed a clear respondent aversion against bacterial and virus-borne mastitis treatment methods. Regardless of such aversions' origins, this insight suggests that treatment strategies involving bacteriophage delivery or gene drives are unlikely to be accepted by farmers as palatable alternatives to current antimicrobial treatments.

Although a majority (60%) of respondents agreed that the government should have a role in assisting the implementation of such treatments, there were distinctly different

preferences in what initiatives would be the most incentivizing. This finding suggests motivation variation based on individual circumstances. The sample size of the study is a limitation on its broader applicability. However, the depth of insight gained sets a strong trajectory for the value of further investigation. To that end, it is recommended that this investigation is replicated on a larger domestic and international scale.

Conclusion

This work is a first step towards understanding how Australian dairy farmers wish to treat mastitis and the significant issue of antimicrobial resistance into the future. The participants demonstrated that they were generally receptive to an alternative, omic-based mastitis treatment option, but that their support was coloured by caution for processor and consumer influence. Notably, respondents showed a clear aversion against bacterial and virus-borne treatment methods e.g. bacteriophages, gene drives, but split opinions on how the government should financially assist in the implementation of such novel, more sustainable management practices. Broader studies should be conducted to verify these insights on a domestic and international scale, so that the research community can invest in the most practical research directions in the fight against antimicrobial resistance.

References (APA 7th)

Kjaerulff, U. B., & Madsen, A. L. (2008). *Bayesian networks and influence diagrams: a guide to construction and analysis*. New York, NY: Springer New York.

Korb, K. B., & Nicholson, A. E. (2010). *Bayesian artificial intelligence*. Boca Raton, Florida: CRC Press.

Morgan, D. L., & Nica, A. (2020). Iterative Thematic Inquiry: A New Method for Analyzing Qualitative Data. *International Journal of Qualitative Methods*, *19*. doi.org/10.1177/1609406920955118.

Molineri, A.I., Cecilia Camussone, C., Zbrun, M.V., Archilla, G.S., Cristiani, M., Neder, V. Calvinho, L., & Signorini, M. (2021). Antimicrobial resistance of Staphylococcus aureus isolated from bovine mastitis: Systematic review and meta-analysis. *Preventative Veterinary Medicine, 188*, 105261. doi.org/10.1016/j.prevetmed.2021.105261.

Morales-Ubaldo, A. L., Rivero-Perez, N., Valladares-Carranza, B., Velázquez-Ordoñez, V., Delgadillo-Ruiz, L., & Zaragoza-Bastida, A. (2023). Bovine mastitis, a worldwide impact disease: Prevalence, antimicrobial resistance, and viable alternative approaches. *Veterinary and Animal Science, 21*, 100306. doi.org/10.1016/j.vas.2023.100306.

Morse, J.M., & Niehaus, L. (2009). *Mixed method design: Principles and procedures*. Walnut Creek, CA: Left Coast Press.

Naranjo-Lucena, A., & Slowey, R. (2023). Invited review: Antimicrobial resistance in bovine mastitis pathogens: A review of genetic determinants and prevalence of resistance in European countries. *Journal of Dairy Science, 106(1)*, 1-23. doi.org/10.3168/jds.2022-22267.

Sensitivity in *S. agalactiae* surveillance on BMT and individual samples

Michael Farre[1*], Malene Vesterager Byskov[1], Lærke Boye Astrup[1]

[1] SEGES Innovation, Århus, Denmark

***Corresponding author's email: mifa@seges.dk**

Introduction

Streptococcus agalactiae (S. agalactiae) is a highly contagious mastitis pathogen that continues to present a major challenge in dairy herd health management. Predominantly transmitted during milking via contaminated equipment, hands, or milking units, this pathogen establishes persistent intramammary infections that elevate bulk tank somatic cell counts (SCC), reduce milk quality, and contribute to significant economic losses (Skarbye et al., 2021). Unlike environmental mastitis pathogens, S. agalactiae is uniquely adapted to the udder environment, facilitating silent, subclinical spread within herds. Additionally, inter-herd transmission routes remain a concern, particularly which challenge the target of maintaining S. agalactiae-free herd status. Strengthening biosecurity protocols and identifying critical control points are essential to mitigate the risk of reintroduction (Churakov et al., 2021).

Active surveillance is vital for early detection both on herd and national level, effective intervention, and long-term control. It enables informed decisions on treatment, how to handle selective dry cow therapy, culling, and the assessment of control program efficacy. Surveillance also provides critical data to track infection dynamics and support targeted management.

Material & methods

The BTM surveillance program functions as a population-based screening tool designed to detect *S. agalactiae* in dairy herds supplying milk to Danish processors and is mandatory according to national regulations. The analysis was based on data extracted from the Danish Cattle Database, which contains PCR results from the biannual bulk

tank milk (BTM) screening for *S. agalactiae*, and records of identification obtained from PCR analyses of individual milk samples collected during Dairy Herd Improvement (DHI), from cows near dry-off, or from acute clinical cases of mastitis. By comparing BTM results with individual cow-level test outcomes, the study applied key epidemiological principles of diagnostic test evaluation — specifically, the estimation of sensitivity — under field conditions. Herds were considered the epidemiological unit, as the primary objective of surveillance was to classify herds according to infection status rather than to identify individual infected animals. To ensure temporal comparability between diagnostic methods, only herds with both BTM and individual cow testing performed within one month before or after the BTM sampling date were included in the analysis. The diagnostic performance of BTM testing for detecting *S. agalactiae* was evaluated using results from the nationwide BTM surveillance conducted in spring 2025, during which samples were collected from all herds shipping milk to Danish processors. As testing of individual cow samples for *S. agalactiae*, either from cows near dry-off or with clinical mastitis is mandatory in Denmark, this approach minimized selection and temporal biases. Furthermore, longitudinal data from July 2024 to June 2025 were analyzed to describe infection dynamics and to evaluate the impact of incorporating individual cow sample results on herd-level surveillance, thereby illustrating both temporal and spatial trends in the national epidemiology of *S. agalactiae*.

Results & Discussion

A total of 2,029 dairy herds were included in the study, of which 739 herds had individual cow samples collected within one month before or after the bulk tank milk (BTM) sampling date. This overlap allowed for direct comparison between herd-level BTM results and individual cow-level test outcomes. *S. agalactiae* was detected in 97 of the 739 herds based on BTM analysis, while 642 herds tested negative. Among these BTM-negative herds, *S. agalactiae* was subsequently identified in 16 herds through individual cow samples analyzed either by PCR or bacteriological culture, indicating the presence of undetected infections at the herd level.

When these 16 herds were considered false negatives, the estimated sensitivity of the BTM-based surveillance method for detecting *S. agalactiae* in Danish dairy herds was 85.8%. This result suggests that while BTM surveillance provides a robust and efficient screening approach, a small proportion of infected herds may remain undetected due to low within-herd prevalence or intermittent bacterial shedding. Such limitations are consistent with previous findings that herd-level sensitivity depends on the proportion of infected cows and bacterial load in the milk sample.

Table 1. Detection of *S. agalactiae* in BTM samples and individual cow samples during spring 2025.

	No *S. agalactiae* in individual samples	*S. agalactiae* in individual samples
No *S. agalactiae* in BTM	626	16
S. agalactiae in BTM	46	51

Between July 2024 and June 2025, a total of 71 milk-producing herds changed status from “Not infected” to “Infected.” Of these, 27 herds were classified as infected based

on detection of S. agalactiae in individual cow samples either by PCR or bacteriological analysis, while 11 herds changed status following detection in BTM samples. The remaining herds became positive through other testing pathways or temporal overlaps between the two diagnostic sources.

These results highlight the complementary roles of BTM and individual cow testing in maintaining an effective national surveillance system. While BTM testing enables efficient herd-level screening across the entire dairy population, inclusion of individual cow testing provides additional sensitivity, particularly for herds with low infection prevalence or subclinical cases. Longitudinal monitoring of herd status changes from 2014 to 2025 further supports the importance of integrating both testing strategies to ensure early detection and to prevent reintroduction of S. agalactiae into previously negative herds.

Conclusion

This study demonstrates that bulk tank milk (BTM) testing provides a method for herd-level surveillance of *S. agalactiae* in Danish dairy herds, with an estimated sensitivity of 85.8%. While BTM analysis effectively identifies most infected herds, incorporating individual cow sample results enhances the overall detection capacity and supports early identification of emerging infections. The findings highlight the value of integrating voluntary individual cow testing with national surveillance programs to improve monitoring accuracy and prevent undetected persistence or reintroduction of S. agalactiae. Continued focus on systematic testing and herd-level biosecurity remains essential to sustain control and progress toward national eradication goals.

References

Churakov, M., Katholm, J., Rogers, S., Kao, R. R., & Zadoks, R. N. (2021). Assessing potential routes of Streptococcus agalactiae transmission between dairy herds using national surveillance, animal movement data and molecular typing. *Preventive Veterinary Medicine*, *197*, 105501. https://doi.org/10.1016/j.prevetmed.2021.105501

Skarbye, A. P., Krogh, M. A., Denwood, M., Bjerring, M., & Østergaard, S. (2021). Effect of enhanced hygiene on transmission of Staphylococcus aureus, Streptococcus agalactiae, and Streptococcus dysgalactiae in dairy herds with automatic milking systems. *Journal of Dairy Science*, *104*(6), 7195–7209. https://doi.org/10.3168/jds.2020-19635

Cross-sectional pilot field study on *Streptococcus uberis*'s mastitis in the Tessin region, Switzerland

Alicia Romano'[1*], Laura Vazquez Rojo[1], Michael Vaccani[2], Lorenzo Sesso[2], Patrizia Riva Scettrini[3], Jérôme Widmer[1], Lotti Egger[1], and Hans Ulrich Graber[1]

[1]Agroscope, Bern, Switzerland.

[2]Repubblica e Cantone Ticino, Ufficio del Veterinario Cantonale, Bellinzona, Switzerland.

[3]Repubblica e Cantone Ticino, Ufficio della Consulenza Agricola, Bellinzona, Switzerland.

***Corresponding author's email: alicia.romano@agroscope.admin.ch**

Introduction

Bovine mastitis is a significant disease affecting dairy cattle worldwide, characterized by inflammation of the mammary gland due, in most cases, to bacterial infections. It leads to reduced milk production, altered milk composition, and substantial economic losses for farmers and the dairy industry (Ruegg 2017). In Switzerland alone, 129.4 million Swiss francs are lost each year due to issues related to mastitis (Heiniger et al., 2014). *Streptococcus uberis* (*S. uberis*) is one of the main mastitis's pathogens, classified as "environmental" pathogen associated with single quarter infection, but based on previous research, a contagious nature was also described (Zadoks et al., 2003).

To investigate the epidemiology and circulation of different subtypes of *S. uberis* in dairy herds, milk and body samples from cows, as well as environmental samples, were collected from 10 voluntary farms in the Canton of Tessin. The samples were analyzed for the presence of *S. uberis*, along with its antimicrobial resistance (AMR) profile and subtypes. Furthermore, the associated inflammatory response in the mammary gland was assessed by evaluating Somatic Cell Count (SCC) and Differential Somatic Cell Count (DSCC).

Material & methods

The study was conducted between April and May 2025, prior to the cows being sent to common alpine pasturing. A total of 260 cows were involved. From these, 1,048 aseptic quarter milk samples were collected. Furthermore, random bedding samples (at least

one sample per every ten cow beds) were collected from all farms. Additionally, swabs from the teats and perineal region of cows were collected in five of the herds.
Milk samples were cultured on blood agar and on a selective chromogenic medium (CHROMagar™ Streptococcus) designed for streptococci identification. For bedding samples, 10 g of material was enriched in 90 ml of Brain Heart Infusion (BHI) broth, incubated for 12 hours, and then streaked onto blood agar and CHROMagar. After 24 hours of incubation, colonies with distinct morphologies were selected for bacterial identification using MALDI-TOF analysis.
From 146 strains isolated from both milk and environmental samples, genotyping was performed using Random Amplification of Polymorphic DNA (RAPD) to compare strains from different sources. A minimum of four colonies per sample type (when available) were analyzed (Schmitt-Van de Leemput & Zadoks, 2007).
SCC and DSCC were measured using a flow cytometry-based method (FACS) as described by Widmer et al. (2022). This analysis was performed on all quarter milk samples from cows that were positive for *S. uberis* (N=67). Quarters from the same cows that were negative for *S. uberis* were used as negative controls (N=89). Furthermore, all microbiologically positive *S. uberis* milk samples were confirmed by PCR targeting the *pauA* gene (Raemy et al., 2013).
To investigate antimicrobial resistance, strains isolated from different environmental sources from two farms were compared using Minimum Inhibitory Concentration (MIC) testing with the commercial MICroSTREP plus Panel Type 6 (Beckman Coulter), which includes 23 antibiotics. Further analysis, including IR Biotyper and Whole Genome Sequencing (WGS), is planned to test more strains for phenotypic and genotypic antimicrobial resistance.

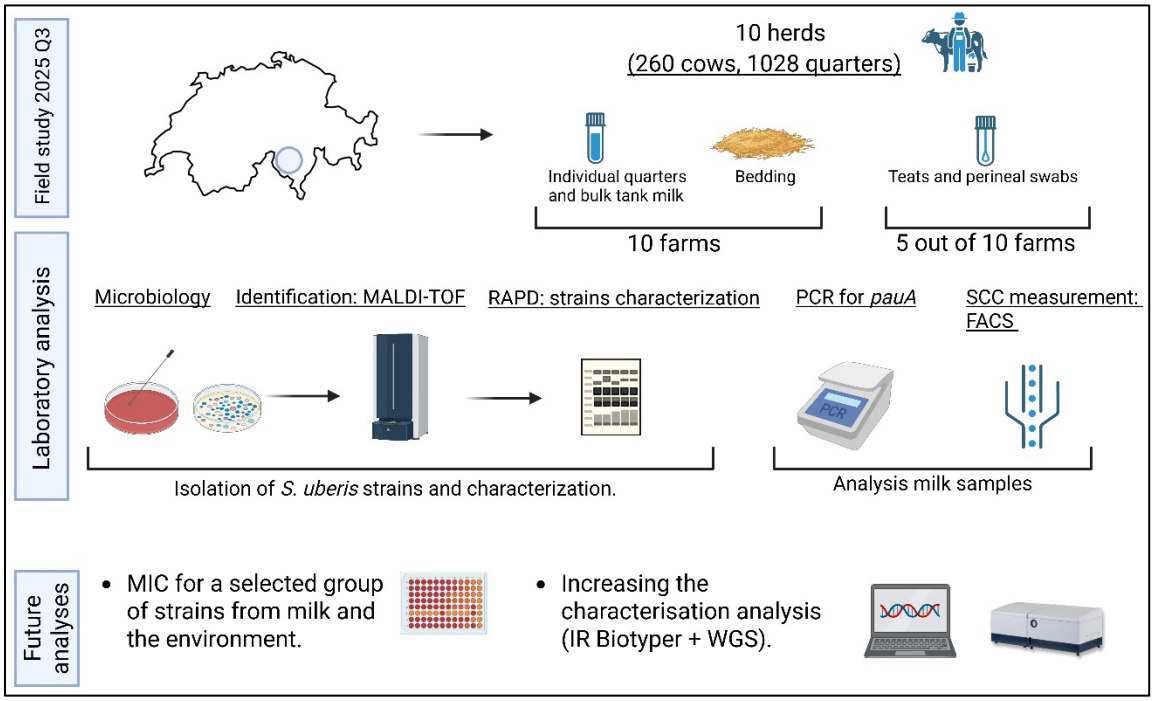


Figure 1: Graphical representation of the research project performed in the Swiss region of Tessin (Created by Biorender).

Results & Discussion

The study revealed that 8 out of the 10 farms were positive for *S. uberis*. The prevalence ranged from 5% to 25.8% at the cow level and from 1.3% to 13.2% at the quarter level.
Body sampling indicated a low prevalence of the pathogen on teat skin (1/129 samples; 0.7%) and the perineal region (6/127 samples; 4.7%). This suggests that these specific body sites are not likely primary reservoirs for *S. uberis* mastitis in these herds. In contrast, bedding materials were a significant source, with 15 out of 36 samples (41.6%) testing positive for *S. uberis*. The bedding samples comprised of 29 straw, 4 straw pellets, and 3 manure-based samples.
SCC in *S. uberis*-positive quarters was highly elevated, with a median of 1.06×10^6 cells/mL, and varied widely, from 1.85×10^3 to 7.31×10^6 cells/mL. Differently, SCC in control quarters (negative to *S. uberis*) showed a median of $1.85 * 10^5$, with a range from 1.54×10^3 to 4.52×10^6 cells/mL. A p-value < .001 showed statistical significance between the two groups of milk samples.
Genotypic characterization using RAPD demonstrated that multiple *S. uberis* genotypes could be isolated from a single quarter, reinforcing existing knowledge about the high strain diversity involved in mastitis pathogenesis (Käppeli et al., 2019).
Preliminary MIC data from two farms revealed distinct AMR patterns: strains isolated from milk samples shared a common AMR profile, which distinctly differed from the profile observed in strains isolated from environmental samples. These analyses will be expanded to all remaining farms to corroborate this observed discrepancy.

Conclusion

Our study demonstrates that teat skin and manure (perineal samples) are not primary reservoirs for *S. uberis* mastitis and confirms bedding as a major reservoir for *S. uberis*. The lack of direct strain overlaps between bedding and milk samples, coupled with the high strain diversity found within quarters, underscores the complexity of transmission routes. These findings highlight the need for rapid, high-resolution genotyping methods, such as IR-Biotyper, to handle the large number of strains required for a precise and meaningful evaluation of *S. uberis* epidemiology. Furthermore, whole genome sequencing is needed to study the genetic differences among genotypes and their virulence factors.

References

Heiniger, D., van den Borne, B. H., Lechner, I., Tschopp, A., Strabel, D., Steiner, A., & Meier, H. (2014). Kosten-Nutzen-Analyse einer Intervention zur Verbesserung der Eutergesundheit in Schweizer Milchviehbetrieben [Cost-benefit analysis of an intervention to improve udder health in Swiss dairy farms]. Schweizer Archiv fur Tierheilkunde, 156(10), 473–481. https://doi.org/10.1024/0036-7281/a000634.

Käppeli, N., Morach, M., Zurfluh, K., Corti, S., Nüesch-Inderbinen, M., & Stephan, R. (2019). Sequence Types and Antimicrobial Resistance Profiles of Streptococcus uberis Isolated From Bovine Mastitis. Frontiers in veterinary science, 6, 234. https://doi.org/10.3389/fvets.2019.00234

Raemy, A., Meylan, M., Casati, S., Gaia, V., Berchtold, B., Boss, R., Wyder, A., & Graber, H. U. (2013). Phenotypic and genotypic identification of streptococci and related bacteria isolated from bovine intramammary infections. Acta veterinaria Scandinavica, 55(1), 53. https://doi.org/10.1186/1751-0147-55-53.

Ruegg P. L. (2017). A 100-Year Review: Mastitis detection, management, and prevention. Journal of dairy science, 100(12), 10381–10397. https://doi.org/10.3168/jds.2017-13023.

Schmitt-Van de Leemput, E., & Zadoks, R. N. (2007). Genotypic and phenotypic detection of macrolide and lincosamide resistance in Streptococcus uberis. Journal of dairy science, 90(11), 5089–5096. https://doi.org/10.3168/jds.2007-0101.

Widmer, J., Descloux, L., Brügger, C., Jäger, M. L., Berger, T., & Egger, L. (2022). Direct labeling of milk cells without centrifugation for counting total and differential somatic cells using flow cytometry. Journal of dairy science, 105(11), 8705–8717. https://doi.org/10.3168/jds.2022-22038.

Zadoks, R. N., Gillespie, B. E., Barkema, H. W., Sampimon, O. C., Oliver, S. P., & Schukken, Y. H. (2003). Clinical, epidemiological and molecular characteristics of Streptococcus uberis infections in dairy herds. Epidemiology and infection, 130(2), 335–349. https://doi.org/10.1017/s095026880200822.

Real-Time PCR in Mastitis Diagnostics

Real-Time PCR in mastitis diagnostics – learnings from Finland

Heidi Hiitiö [1]

[1] Pinehill Goblin Oy, Nurmijärvi, Finland

***Corresponding author's email: Heidi.hiitio@pinehillgoblin.fi**

Introduction

Mastitis remains the most frequent and costly disease of dairy cattle. Accurate, rapid etiological diagnosis is essential for targeted therapy, antimicrobial stewardship, and herd-level control. Since June 2010, Finland has implemented routine real-time multiplex PCR (qPCR) for mastitis diagnostics at national scale, replacing most bacteriological culture (BC) and generating a unique, longitudinal dataset to inform udder-health management.

This large-scale adoption provides a unique basis for combining diagnostic, milk quality, and antimicrobial use data to evaluate udder health and management outcomes at population level.

Material & methods

We summarize operational experience from Finland's centralized diagnostic workflow involving Valio Oy's laboratory network, Movet IDEXX, and the University of Helsinki, which collectively process over 130,000 mastitis PCR samples annually, compared with approximately 2,000 bacterial culture (BC) samples analyzed mainly at the University of Helsinki and Movet.

Sample frames include aseptically collected quarter samples from clinical and subclinical mastitis cases, taken predominantly by farmers under veterinary guidance. In addition, national Dairy Herd Improvement (DHI) data from over 250,000 cows are analyzed monthly for somatic cell count (SCC), allowing integration of herd-level inflammation and infection trends with PCR pathogen profiles.

PCR analyses employ commercial qPCR panels (12–16 targets including β-lactamase resistance gene). All laboratories follow harmonized procedures for aseptic sampling, DNA extraction, amplification, and automated Ct-based result interpretation.

Antimicrobial use data originate from FINRES-Vet national surveillance, enabling linkage of diagnostic adoption with trends in veterinary antimicrobial consumption.

Results & Discussion

Annual qPCR throughput exceeds 130,000 samples, demonstrating the national scale and robustness of Finland's udder-health surveillance system. The median turnaround time decreased from ≥ 48 h for BC (and 6–10 days for Mycoplasma bovis) to same-day reporting (~4 h analytical time). Across multiple Finnish and international studies, qPCR consistently detected mastitis pathogens more frequently than BC and achieved pathogen detection rates of 83–92 % in routine diagnostics.

Sample quality was acceptable at large scale: ~13 % classified as contaminated (> 2 targets), and 12–18 % PCR-negative, indicating reliable farmer sampling. National DHI SCC data show a gradual decline in herd-average SCC over the past decade, corresponding with enhanced diagnostic coverage and targeted herd management. Importantly, antimicrobial usage in Finnish dairy herds has decreased steadily since 2010, contrary to initial concerns that PCR adoption would increase prescriptions. According to FINRES-Vet 2022, both overall antimicrobial consumption and resistance indicators declined, supporting improved stewardship outcomes.

Conclusion

Finland's nationwide qPCR implementation demonstrates that molecular diagnostics can be successfully integrated into large-scale udder-health management without increasing antimicrobial use. The key lessons include:

1. Embedding diagnostics within herd-health strategy - clear sampling indications, and aseptic technique.
2. Combining molecular and production data - integration of PCR results with DHI SCC databases enables herd-specific pathogen profiling and longitudinal benchmarking.
3. Interpreting Ct values contextually - Ct data should be routinely reported and evaluated alongside SCC and clinical findings.
4. Monitoring stewardship metrics—routine PCR use facilitates evidence-based antimicrobial decision-making, reducing unnecessary treatments.

Finland's experience shows that qPCR, when combined with systematic SCC monitoring and national antimicrobial surveillance, forms a powerful, objective, and data-driven foundation for precision mastitis management. The Finnish model provides an international benchmark for linking diagnostics, prevention, and responsible medicine use in sustainable dairy production.

References

Halasa, T., Huijps, K., Østerås, O. & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. Veterinary Quarterly 29, 18–31.

Heikkilä, A.-M., Nousiainen, J. I. & Pyörälä, S. (2012). Costs of clinical mastitis with special reference to premature culling. Journal of Dairy Science 95, 139–150.

Seegers, H., Fourichon, C. & Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cakle herds. Vet. Res. 34, 475–491 (2003).

Sguizzato, A. L. L. et al. (2024). Understanding the dynamics of mastitis in milk yield: decoding onset and recovery pakerns in response to mastitis occurrence. JDS Communications. https://doi:10.3168/jdsc.2024-0579.
Williamson, J., Callaway, T., Rollin, E. & Ryman, V. (2022). Association of Milk Somatic Cell Count with Bacteriological Cure of Intramammary Infection—A Review. Agriculture 12, 1437.
Advanced Techniques in Diagnostic Microbiology. (Springer US, Boston, MA, 2013). doi:10.1007/978-1-4614-3970-7.
Mahmmod, Y. S., Klaas, I. C. & Enevoldsen, C. (2017). DNA carryover in milk samples from routine milk recording used for PCR-based diagnosis of bovine Staphylococcus aureus mastitis. Journal of Dairy Science 100, 5709–5716.
Taponen, S., Salmikivi, L., Simojoki, H., Koskinen, M. T. & Pyörälä, S. (2009). Real time polymerase chain reaction-based idenitfication of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. Journal of Dairy Science 92, 2610–2617.
Shah, K., Nauriyal, D. & Joshi, C. (2018). Real-time PCR Based Assay Offers High Resolution in Detecting Bacterial Species from Bovine Sub-clinical Mastitis for Prudent Antimicrobial Treatment. J PURE APPL MICROBIO 12, 887–895.
Koskinen, M. T. et al. (2010). Field comparison of real-%me polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. Journal of Dairy Science 93, 5707–5715.
Spikel, S. & Hoedemaker, M. (2012). Mastitis diagnosis in dairy cows using PathoProof real-time polymerase chain reaction assay in comparison with conventional bacterial culture in a Northern German field study. Berliner und Münchener tierärztliche WochenschriR 125, 494–502.
Hiitiö, H. (2018) Multiplex Real-Time PCR in Bovine Mastitis Diagnostics. Academic Dissertation 2018.
Vakkamäki, J., Taponen, S., Heikkilä, A.-M. & Pyörälä, S. (2017). Bacteriological etiology and treatment of mastitis in Finnish dairy herds. Acta Vet Scand 59, 33.
Bradley, A. J., Leach, K. A., Breen, J. E., Green, L. E. & Green, M. J. (2007). Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Veterinary Record 160, 253–258.
Koivula, M., Pitkälä, A., Pyörälä, S. & Mäntysaari, E. A. (2007). Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland. Acta Agriculturae Scandinavica, Section A - Animal Science 57, 89–96.

Genetic typing and *in-vitro* adherence profiles of *Pseudomonas aeruginosa* isolates from intramammary infections and bulk tank milk origins

Marcos Munoz[1*] and Brandon Gonzalez-Cordova[1]
[1]Universidad de Concepcion, Chillan, Chile
***Corresponding author's email: marcomun@udec.cl**

Introduction

Pseudomonas aeruginosa is an environmental and opportunistic organism of importance in the quality of bulk tank milk (BTM), but also for udder health of dairy cows. Epidemiology of *P. aeruginosa* in dairy operations, sources and reservoirs include drinking water, bedding, and contaminated surfaces. Whereas for *P. aeruginosa* clinical mastitis (CM) and subclinical intramammary infections (IMI), udder cleaning towels, contaminated dipping, contaminated antibiotic preparations, and milking machine biofilms have been reported as sources (Daly et al., 1999; Schauer et al., 2021; Gagnon et al., 2025). Given the variety of sources and environmental reservoirs for *P. aeruginosa*, appropriate surface hygiene and sanitization, milking procedures, and proper antibiotic use and handling are considered key preventive measures. Despite of this previous work and the advancement in diagnosis for IMI, a not-clear understanding of the epidemiology, and the dynamics of *P. aeruginosa* IMIs in lactating dairy cows still exists. In this study we report *P. aeruginosa* subclinical IMIs of likely contagious transmission; and compared in-vitro adherence profiles of isolates from IMI and those of BTM origin.

Material & methods

Pseudomonas aeruginosa Isolates from intramammary infections (IMI) were isolated from 2 subclinical cases occurring in different quarters of the same cow, 3 weeks apart. Namely, B04 and B05. The 2 IMI isolates belonged to a herd from the southern Los Rios Region of Chile. In addition, 7 unrelated *P. aeruginosa* isolates were obtained from the culture of bulk tank milk (BTM) samples. Each isolate belonged to a different dairy operation from the central-southern region of Chile. Namely, B79, B80, B83, B85, B88, B89, and B92 isolates. Genetic molecular typing of isolates was done using RAPD-PCR testing, using the methods described by Munoz and Zadoks (2007). *Pseudomonas*

aeruginosa ATCC 15442, and Klebsiella pneumoniae ATCC 13883, were used as a positive and negative control, respectively. Molecular banding patterns were read and classified by two independent observers. On the other hand, *in-vitro* adherence profiles of all *P. aeruginosa* isolated were evaluated using a consensus Microtiter Plate Assay (MPA) method. Namely, all 9 *P. aeruginosa* and positive and negative-control strains were evaluated by their ability to form adherences in laboratory conditions, using consensus MPA methods from previous reports by different authors (Peeters et al., 2007; Massé et al 2020; Fidelis et al., 2024). Briefly, tested isolates and control strains were inoculated in 10 mL trypticase soy broth, incubated at 37 C for 18 h. Each one was processed and transferred into 8 wells of polystyrene flat bottom microtiter plates (MPA). Plates were incubated, processed, stained with 1% crystal violet, and adherence optical density (OD) was evaluated using an EPOCH® spectrophotometer at 590 nm. Finally, the average OD of isolates, processed in duplicates, was calculated by subtracting the OD average blank control. Analyses of results was done using descriptive statistics methods. Whereas diversity of isolates of different epidemiological origin was evaluated using Simpson Index of Diversity (SID). Statistical differences with p- values < 0.05 were considered significant.

Results & Discussion

Molecular testing

Results of RAPID DNA typing yielded the 2 *P. aeruginosa* subclinical IMI isolates having indistinguishable banding patterns type (Type A). These two events occurred 3 weeks apart in different quarters of the same cow. Thus, suggesting contagious transmission between quarters. On the other hand, BTM *P. aeruginosa* isolates, each one originating from one of the 7 epidemiologically unrelated dairy farms, yielded 7 unique molecular banding pattern fingerprints (namely, Type B, C, D, E, F, G, and H). Calculated Simpson Index of Diversity for all isolates was 0.97. Supporting the notion of a large genetic diversity of *P. aeruginosa* isolates in BTM and agreement with the findings of Gagnon et al. (2025).

In-vitro adherence MPA testing

Microtiter plate assay in-vitro adherence profiles of BTM isolates show a wide range of adherence patterns (Figure 1). These results agree with the DNA strain-type diversity of the strains; with BTM isolates having from “low” adherence ability to a “high” adherence ability (Figure 1). Moreover the 2 type-A fingerprints of the seemingly IMI contagious transmission within the cow, have no-different adherence ability. Interestingly, a higher ability to form an in-vitro adherence does not seem to be fundamental to produce a successful seemingly contagious IMI, indicating that other virulence factors may be involved.

Figure 1. Genetic typing and in-vitro adherence profiles of *Pseudomonas aeruginosa* isolates from intramammary infections and bulk tank milk origins from 8 dairy farms.

Strain	Origin	Farm	DNA Strain-type	*In-vitro* adherence OD* avg. value	Adherence Ratio (AR)	*In-vitro* adherence ability**
B04	IMI	1	A	0.153	0.327	Medium
B05	IMI	1	A	0.137	0.279	Medium

B79	BTM	2	B	0.426	0.772	Medium-high
B80	BTM	3	C	0.927	1.110	High
B85	BTM	4	D	0.464	0.809	Medium-high
B86	BTM	5	E	0.072	0.000	Low
B88	BTM	6	F	0.467	0.812	Medium-high
B89	BTM	7	G	0.299	0.618	Medium-high
B92	BTM	8	H	1.556	1.335	High
15442	ATCC	-	-	0.949	1.120	High

IMI: intramammary infection; BTM: bulk tank milk; ATCC: American type culture collection. *OD: optical density (590 nm); *Low AR < 0.037; Medium: AR > 0.037 to 0.420; Medium-high: AR > 0.420 to 1; High: AR > 1

Conclusion

We found a large genetic diversity in BTM *P. aeruginosa* isolates from different herds. DNA RAPID typing discriminatory power demonstrated to be of value to assess *P. aeruginosa* molecular diversity. We report evidence of successful *P. aeruginosa* IMI contagious transmission between different quarters within the same cow. Other virulent factors rather than a high in-vitro adherence ability may also be involved to produce successful IMI and host adaptation in *P. aeruginosa* organisms. Together, this study shows that additional research in both adherence and virulence factors of *P. aeruginosa* should be done.

References

Daly, M., Power, E., Björkroth, J., Sheehan, P., O'Connell, A., Colgan, M., Korkeala, H., y Fanning., S. (1999). Molecular analysis of Pseudomonas aeruginosa: Epidemiological investigation of mastitis outbreaks in Irish dairy herds. Appl Environ Microbiol. 65(6), 2723–2729. DOI: 10.1128/AEM.65.6.2723-2729.1999

Fidelis, C. E., Orsi, A. M., Freu, G., Gonçalves, J. L., & Santos, M. V. D. (2024). Biofilm formation and antimicrobial resistance of Staphylococcus aureus and Streptococcus uberis isolates from bovine mastitis. Veterinary Sciences, 11(4), 170.

Gagnon, M., Jean, S., de Toro-Martín, J., LaPointe, G., Guévremont, É., Dufour, S., & Roy, D. (2025). Insights into the prevalence of Pseudomonadota and yeasts on milking system surface biofilms. Journal of Dairy Science.

Massé, J., Dufour, S., & Archambault, M. (2020). Characterization of Klebsiella isolates obtained from clinical mastitis cases in dairy cattle. Journal of dairy science, 103(4), 3392-3400.

Munoz, M. A., & Zadoks, R. N. (2007). Patterns of fecal shedding of Klebsiella by dairy cows. Journal of dairy science, 90(3), 1220-1224.

Peeters, E., Nelis, H. J., & Coenye, T. (2008). Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of microbiological methods, 72(2), 157-165.

Schauer, B., Wald, R., Urbantke, V., Loncaric, I., y Baumgartner, M. (2021). Tracing Mastitis Pathogens-Epidemiological Investigations of a Pseudomonas aeruginosa Mastitis Outbreak in an Austrian Dairy Herd. Animals. 11(2), 279. DOI: 10.3390/ani11020279

The Antimicrobial Usage Practices of Michigan and Wisconsin Dairy Veterinarians Regarding the Treatment and Prevention of Mastitis

Jaimie M. Strickland[1*], Jean Kayitsinga[1], Maria Jose Fuenzalida Valenzuela[2], Pamela L. Ruegg[1]
Michigan State University[1], East Lansing, United States
University of Wisconsin, River Falls[2], United States
***Corresponding author's email: strick51@msu.edu**

Introduction

Intramammary treatments for mastitis account for 78% of antimicrobial use in adult dairy cows (de Campos, J. L. et al., 2021). Veterinarians are key partners in improving milk quality and reducing antimicrobial use on dairies through mastitis prevention strategies, implementing selective mastitis treatment protocols, and selective dry treatment programs. However, veterinary shortages may prevent the time or resources required for veterinarians to provide all those services. Our objective was to evaluate the practices of dairy veterinarians in MI and WI regarding their client's milk quality and related antimicrobial usage (AMU).

Material & methods

This study was approved by the Michigan State University Biomedical and Health Institutional Review Board (STUDY00011829). Our sampling frame was identified using the member directory from AABP. The survey instrument was developed to include questions regarding 1) practice characteristics, 2) prescribing practices and volume of drug sales, 3) current antimicrobial usage practices on client farms, and 4) demographics of respondent. Surveys were implemented using Qualtrics (Seattle, WA) and were pre-tested by 2 dairy veterinarians in academia. Postcards were mailed to Michigan (N = 71) and Wisconsin (N = 254) 1 week prior to the survey. Reminder emails were sent every 2 weeks for a total of 3 reminder emails, and a reminder postcard was mailed 4 weeks after the initial email. The descriptive analysis was performed using SPSS Inc. (version 29, Chicago, IL). Fisher's exact test was performed due to the small sample size. Ordinal logistic regression models were created for recommended veterinary practices using the generalized linear model option. Backwards selection was used using an α of 0.05. P values <0.05 were considered significant.

Results & Discussion

Of the 325 vets contacted, 85 surveys were fully completed for a 26% response rate. Gender was evenly distributed among females (47%) and males (53%). The distribution of ages was ≤39 years (34%), 40-59 years (40%), and ≥60 years (26%). Among respondents, the distribution of employment status was owner/partners (47%), associates (21%), solo practitioners (22%), and others (which included consultants and farm veterinarians) (10%). Employment status was associated with age, providing prescriptions for mild or moderate mastitis, the creation of treatment protocols for severe mastitis, offering in-clinic milk culture, and the number of times milk culture results were used to make treatment decisions in the previous 12 months ($P < 0.05$; Table 1). A high proportion of associate vets were ≤39 years which is expected for that role. Fewer vets, except for associates, prescribed antibiotics for mild or moderate mastitis than severe mastitis. However, when it came to writing treatment protocols, 75% and 100% of owner/partners and other veterinarians created protocols for 76-100% of their clients, whereas approximately half of associates and solo practitioners created treatment protocols for ≤75% of their clients. The distribution of treatment protocols was nearly identical for mild or moderate and severe mastitis. Only 10% of solo practitioners offer in-clinic milk culture services, which may be because they are less likely to own the required equipment such as an incubator, or they might not have suitable laboratory space. In the ordinal regression for mild/moderate treatment protocols an increased number of requirements for a valid veterinary-client-patient-relationship (VCPR) was associated with an increased odds of having written protocols for 76-100% of dairy clients ($P = 0.023$; Table 2). However, having veterinarians that didn't work for the practice writing prescriptions was associated with a decreased odds of having protocols for 76-100% of clients ($P = 0.004$). There was a tendency for veterinarians who spent 75% or more of their professional time on dairy cows to have written mild/moderate mastitis protocols for 76-100% of clients. Interestingly, employment type wasn't significant in this model. Utilizing milk culture data and having treatment protocols are essential to advise producers on selective treatment of clinical mastitis and improving overall milk quality on dairy farms (Lago, A. et al., 2011). Overall, solo practitioners reported using fewer recommended practices. Veterinarians that did not have a robust VCPR with their clients or weren't involved with writing prescriptions for their clients had decreased odds of writing treatment protocols for mild/moderate mastitis.

Table 1: Characteristics and Milk Quality Engagement of Michigan and Wisconsin Veterinarians

Employment type characteristics	Owner/ Partner	Associate	Solo Practitioner	Other	P value
	N (%)	N (%)	N (%)	N (%)	
Age groups					
≤39	10 (25)	13 (72.2)	3 (15.8)	3 (37.5)	<0.01
40-59	23 (57.5)	1 (5.6)	9 (47.4)	1 (12.5)	
≥60	7 (17.5)	4 (22.2)	7 (36.8)	4 (50)	

Prescribed antibiotics for:					
Mild/Moderate mastitis	27 (67.5)	17 (94.4)	9 (47.4)	5 (62.5)	0.014
Severe mastitis	39 (97.5)	17 (94.4)	18 (94.7)	7 (87.5)	0.331
Created protocols for __% of clients					
Mild/Moderate mastitis					
0-25%	4 (10)	5 (29.4)	7 (36.8)	0 (0)	0.059
26-75%	6 (15)	4 (23.5)	2 (10.2)	0 (0)	
76-100%	30 (75)	8 (47.1)	10 (52.6)	8 (100)	
Severe mastitis					
0-25%	3 (7.5)	5 (29.4)	7 (36.8)	0 (0)	0.048
26-75%	7 (17.5)	4 (23.5)	2 (10.5)	1 (12.5)	
76-100%	30 (75)	8 (47.1)	10 (52.6)	7 (87.5)	
Recommends selective dry cow therapy in appropriate herds	28 (70)	17 (94.4)	13 (68.4)	7 (87.5)	0.129
Offers in-clinic milk culture	30 (75)	12 (66.7)	2 (10.2)	5 (62.5)	<0.01
Times milk cultures were used to make treatment decisions in the last 12 months					
0-1	4 (10)	3 (16.7)	11 (57.9)	3 (37.5)	<0.01
2-5	10 (25)	3 (16.7)	5 (26.3)	1 (12.5)	
5+	26 (65)	12 (66.7)	4 (21.1)	7 (87.5)	

Table 2: Ordinal regression model for % of clients that veterinarian created mild/moderate mastitis treatment protocols for

Parameter		Odds Ratio	95% Confidence Interval		Sig.
			Lower	Upper	
Created protocols for __% of clients for mild/moderate mastitis	76-100% (Referent)				
	26-75%	2.723	0.254	29.191	0.408
	0-25%	0.949	0.090	10.028	0.965
Age groups		0.770	0.385	1.539	0.459
Average client herd size		1.207	0.862	1.690	0.273
Clinic gross revenue		1.210	0.859	1.704	0.275
Employment type		0.929	0.530	1.630	0.799
≥75% Professional time - dairy cows		3.173	0.973	10.346	0.055
Veterinarians outside practice write prescriptions		0.167	0.049	0.573	0.004
Number of items required for VCPR		1.364	1.043	1.783	0.023

Conclusion

There are opportunities to improve practices around milk quality and AMU on dairy farms. Veterinarians are essential in optimizing AMU around mastitis, but are lacking in

key areas. Further data analysis will be completed in time to present at the conference if this abstract is accepted.

References

de Campos, J. L., A. Kates, A. Steinberger, A. Sethi, G. Suen, J. Shutske, N. Safdar, T. Goldberg, and P. Ruegg. 2021. Quantification of antimicrobial usage in adult cows and preweaned calves on 40 large wisconsin dairy farms using dose-based and mass-based metrics. Journal of Dairy Science 104:4727-4745.

Lago, A., S. Godden, R. Bey, P. Ruegg, and K. Leslie. 2011. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. Journal of dairy science 94:4441-4456.

Evaluation of an automatic image classifier for analysis of bacterial growth on a multiple-agar plate developed for bovine mastitis

Lena-Mari Tamminen[1], Josef Dahlberg[1*]

[1] Swedish University of Agricultural Science, Uppsala Sweden

***Josef_Dahlberg@slu.se**

Introduction

Identification of mastitis causing bacteria is in many aspects crucial, if done rapidly it can be used as a decision tool for selective treatment of clinical cases and even if it is not done rapidly it provides important information about pathogens circulating in the herd. Clinical mastitis is, in Sweden and many other countries, the main reason for antibiotic treatment of dairy cows(Växa, 2023). Even though antibiotic treatment often is beneficial for a cow suffering from clinical mastitis it is not always justified. A number of mulitple-media agar plates have been developed of easy and rapid diagnosis of mastitis causing pathogens. Automatic image analysis has in later years become applicable to a wide range of subjects and fields. In mastitis diagnostics, an AI-based automatic image analysis has been used to identify clinical mastitis-causing pathogens on chromogenic multiple-agar plates (Garcia, Martins, Porto, Nobrega, & Dos Santos, 2024). A bacterial classifier, Bacticam, for automatic reading and classification of bacterial growth on SELMA + multiple-agar plates has been developed by the company Agricam (Linköping, Sweden). The bacterial classifier is based on an artificial neural network doing automatic image analysis of the multiple-media agar plates. The Bacticam is developed to be used on farms by trained farm personnel.
The aims of this study were to; 1) evaluate the accuracy of the automatic bacterial classifier compared to a (gold) standard laboratory culture, 2) explore the variation in results related to place of plating and after transportation of samples.

Material & methods

Milk samples from dairy cows with clinical mastitis arriving at the Mastitis Laboratory, at the Swedish Veterinary Agency (SVA) in Uppsala, Sweden, were used and is the main source of data in this study. SVA is a reference laboratory and the Mastitis Laboratory is

accredited according to ISO 17025. At the Mastitis Laboratory milk samples were handled and analysed in parallel, both according to the (gold) standard methods normally applied and by the automatic image classifier. A second source of data was provided by the company Agricam and consisted of image classifier results from on-farm Bacticam stations from milk samples that were also sent to the Mastitis Laboratory. Data analysis was performed on three levels; general performance (i.e. proportion of samples with diagnose), specific performance (sensitivity and specificity for specific diagnoses) and performance related to place (i.e. on-farm vs in-lab vs gold standard analyses).

Results & Discussion

In total 1212 milk samples from cows with clinical mastitis were analyzed by both methods. The automatic image classifier returned a bacterial diagnosis for 70% of the samples while 30% required additional evaluation. After excluding samples with co-infection and multiple bacterial diagnoses per samples, 1077 samples with a single bacterial diagnosis were used for analyses of specific performance. Of the 1077 samples with a single bacterial diagnosis the automatic bacterial classifier could theoretically (based on bacterial diagnosis) match the response to 90% of the samples. In reality, the automatic image classifier matched the response for 71% of the samples. When the bacterial classifier matched the response from the gold standard analysis there was a high specificity for all bacterial diagnoses. The sensitivity varied between the bacterial diagnoses and was high for *E. coli, S. aureus,* Strep. spp and no growth and medium-low for *Klebsiella*, Staph. spp., beta-hemolytic streptococci and mixed infection. Regarding performance related to place of analysis, there was a generally high accordance between samples analyzed with the bacterial classifier on-farm or in lab and the gold standard method. A higher occurrence of mixed flora in samples analyzed with the bacterial classifier on-farm highlight the importance of proper handling of samples and agar plates.

Conclusion

We conclude that the AI-based image analysis bacterial classifier, Bacticam, is showing promise in accurately diagnosing common mastitis pathogens. The classifier performs well with high specificity for all diagnosed pathogens, assuring avoidance of unnecessary treatment. For bacterial diagnoses of *E.coli, S. aureus* and non-haemolytic streptococci the sensitivity of the bacterial classifier is high. An apparent benefit of the bacterial classifier is the reduced time from sampling to results, although operators must take appropriate measures before plating the milk to minimize growth of mixed infection.

References

Garcia, B. L. N., Martins, C., Porto, L. F., Nobrega, D. B., & Dos Santos, M. V. (2024). Accuracy of an AI-based automated plate reading mobile application for the identification of clinical mastitis-causing pathogens in chromogenic culture media. *Sci Rep, 14*(1), 1208. doi:10.1038/s41598-023-50296-w

Växa. (2023). *Djurhälsostatistik 2022-2023*.

Bacteria-dependent modulation of Immune Responses in the Bovine Udder

Alicja Krysmann[1], Ian Woolsey[2], Preben Boysen[2], Davide Porcellato[1*]

1. **Norwegian University of Life Sciences, Faculty of Chemistry, Biotechnology and Food Science, Ås, Norway**
2. **Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Ås, Norway**

***Corresponding author's email: davide.porcellato@nmbu.no**

Introduction

Bovine mastitis remains a major disease affecting dairy herds globally due to its complex and multi-etiological nature. Understanding the interplay between udder microbiota, pathogen persistence, and host immune responses is essential for improving mastitis management. This study integrates multiple approaches to investigate these complex interactions across lactation stages.

Material & methods

Norwegian Red cows were sampled longitudinally across two lactation periods. Quarter-level hindmilk samples were collected at multiple lactation stages to assess udder microbiota and host immune responses. Microbial diversity and pathogen presence were analyzed using 16S rRNA gene amplicon sequencing and shotgun metagenomics. This enabled genome-centric analysis of metagenome-assembled genomes (MAGs), allowing species- and strain-level resolution of the udder microbiota without the need for culturing. Host responses were evaluated through somatic cell count (SCC), flow cytometry, cytokine profiling, and proteomic analysis of milk somatic cells. Proteomic data were further analyzed using weighted gene co-expression network analysis (WGCNA) to identify immune-related protein modules.

Results & Discussion

Temporal shifts in microbial diversity, SCC levels, and profiles were detected throughout lactation, with an increase in pathogen levels and reduced diversity at the end of lactation. The analysis of MAGs identified key functional traits at the species level, including antimicrobial resistance genes and immune evasion systems, enabling detailed characterization of potential pathogenicity. The temporal profiling of the

microbiota identified persistent infections of *Staphylococcus aureus* and *Staphylococcus chromogenes*, with the latter associated with suppressed cytokine levels (IFN-γ, IL-10, TNF-α) and minimal granulocyte recruitment. In contrast, the presence of *S. aureus* and *Streptococcus spp.* triggered elevated SCC response and inflammatory somatic cell profiles, which were not detected in samples enriched with other genera such as *Corynebacterium* and *Lactococcus*. These genera may contribute to maintaining a balanced microbial ecosystem in the absence of inflammation. By applying network analysis on the SCC proteome, we identified modules (groups of co-expressed proteins) that negatively and positively correlated with SCC levels. These modules suggest the activation of specific host regulatory pathways in response to different microbial stimuli and infection dynamics. Further investigation of the proteomes revealed 67 differentially expressed proteins associated with various pathogens, of which 19 were linked to important immune functions such as Toll-like receptor 2 (TLR2) and lactoferrin.

Conclusion

This study highlights the complex interplay between udder microbiota, pathogen persistence, and host immune responses across lactation. The integration of microbiome and proteomic data revealed distinct host regulatory pathways linked to somatic cell dynamics and pathogen-specific responses. These insights advance our understanding of mastitis disease mechanisms and may inform targeted strategies for improving udder health and disease resilience in dairy herds.

Comparative *in vitro* inhibition of mastitis pathogens by probiotic, paraprobiotic, and postbiotic derived from bovine-related non-*aureus* staphylococci and mammaliicocci

Bruno Toledo-Silva[1*], Bram Desmet[1], Kristien Mertens[1], Gregory Rota[1], and Sarne De Vliegher[1]

[1] Ghent University, Merelbeke, Belgium

***Corresponding author's email: Bruno.Silva@UGent.be**

Introduction

Antimicrobial resistance represents a major challenge to both human and animal health and has become a critical concern in the dairy sector, where antimicrobials are extensively used to manage bovine mastitis (Stevens et al., 2016). Increasing restrictions on antibiotic use (European Parliament and Council of the European Union, 2019), combined with the need for sustainable alternatives, have driven the development of non-antibiotic strategies for mastitis prevention and control.

In this context, non-*aureus* staphylococci and mammaliicocci (NASM), particularly *Staphylococcus chromogenes* and *S. simulans*, have been shown to inhibit the growth of major mastitis pathogens such as *S. aureus* and *Streptococcus uberis* (Toledo-Silva et al., 2022). Despite this potential, most studies have focused on the activity of live bacterial cells (probiotics), whereas the antimicrobial properties of their heat-killed preparations (paraprobiotics) and cell-free supernatants (postbiotics) remain largely unexplored.

This study aims to evaluate and compare the *in vitro* antimicrobial activity of probiotics, paraprobiotics, and postbiotics derived from *S. chromogenes* and *S. simulans* isolates against major mastitis pathogens.

Material & methods

Bacterial isolates: NASM isolates were obtained from our repository and previously identified to the species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). Eight NASM isolates were selected based on their distinct inhibitory profiles (Toledo-Silva et al., 2021), comprising two S. *chromogenes* and two *S. simulans* originating from milk, as well as two *S. chromogenes*

and two *S. simulans* originating from teat apex (TA). *S. chromogenes* TA (previously described as inhibitor C2; De Vliegher et al., 2004) will serve as a positive control for the quantitative antimicrobial assay, while *S. aureus* and *S. uberis* strains will be included as target pathogens.

Preparation of probiotic, paraprobiotic, and postbiotic suspensions: For each selected NASM isolate, three preparations were obtained: probiotics, paraprobiotics, and postbiotics suspensions. Probiotic cultures were grown in tryptic soy broth (TSB) until reaching approximately 10^8 CFU/mL. Paraprobiotic and postbiotic preparations were generated from fresh cultures adjusted to the same density. Paraprobiotics was produced by incubating NASM cultures at 60 °C for 60 min, and postbiotics was generated by centrifuging NASM cultures at 3,200 × *g* for 1 hour, followed by filtration through 0.4 µm membranes to remove residual cells. Viability loss was confirmed by absence of growth on Columbia agar with 5% sheep blood after 24 h at 37 °C. Both paraprobiotics and postbiotics were stored at –20 °C until testing.

Quantification of antimicrobial activity: Antimicrobial activity will be quantified using a broth-based co-culture assay adapted from Soundharrajan et al. (2021) and the microdilution format described by the Clinical and Laboratory Standards Institute (CLSI, 2023), with modifications to allow direct co-culture of NASM preparations and target pathogens. For each assay, 100 µL of NASM preparations (probiotic, paraprobiotic, or postbiotic suspensions) previously standardized to 10^8 CFU/mL (or equivalent concentrations for non-viable preparations) will be added to 96-well plates. Subsequently, 100 µL of *S. aureus* or *S. uberis* suspensions (10^2–10^6 CFU/mL) will be added to the same wells, resulting in final pathogen inocula of approximately 10^1–10^5 CFU per well. Each condition will be tested in duplicate across two independent experiments performed on different days. Positive controls will include *S. chromogenes* TA, while negative controls will consist of *S. aureus* or *S. uberis* suspensions mixed with sterile TSB. Plates will be incubated aerobically at 37 °C overnight. Following incubation, co-cultures will be serially diluted in sterile phosphate-buffered saline (PBS), and 10 µL of each dilution will be spotted onto tryptic soy agar (TSA) for CFU enumeration. After 18 h of aerobic incubation at 37 °C, bacterial growth will be quantified by counting droplets containing approximately 3–50 CFU. The inhibitory effect of NASM preparations will be expressed as the log CFU reduction, calculated by comparing *S. aureus* or *S. uberis* counts in co-cultures with those in control wells containing the pathogens alone.

Results & Discussion

At this stage, the preparation of paraprobiotic and postbiotic suspensions has been completed, and quantitative co-culture assays are being optimized. The quantitative assessment is expected to reveal distinct inhibitory profiles across NASM preparations (probiotics, paraprobiotics, and postbiotics), providing insight into the bacterial components primarily responsible for pathogen inhibition. Probiotic preparations are anticipated to exert the strongest inhibition through metabolic activity and competitive exclusion. In contrast, paraprobiotic and postbiotic preparations may retain partial inhibitory activity mediated by stable antimicrobial compounds, supporting their potential as safe and shelf-stable alternatives for mastitis prevention.

Previous findings (Toledo-Silva et al., 2022) also indicated that *S. simulans* and NASM isolates originating from teat apices required lower concentrations to inhibit *S. aureus* growth, suggesting enhanced inhibitory potential of these isolates. In line with these observations, NASM inhibition in the present study is expected to be generally stronger

against *S. aureus* than *S. uberis,* and to vary according to NASM species, strain, and origin.

Conclusion

This study will clarify whether the antimicrobial activity of *S. chromogenes* and *S. simulans* against major mastitis pathogens depends on bacterial viability or on stable bioactive components. Comparative analyses across NASM species and strains, origins, and preparation types will clarify the mechanisms of NASM-mediated inhibition and quantify their antimicrobial effects. Future investigations focusing on metabolite characterization and host immune responses will further advance the development of NASM-based probiotic, paraprobiotic, and postbiotic strategies as sustainable, non-antibiotic tools for bovine mastitis prevention and control.

References

Clinical and Laboratory Standards Institute. (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed.; CLSI supplement M100). Clinical and Laboratory Standards Institute.

De Vliegher, S., Opsomer, G., Vanrolleghem, A., Devriese, L. A., Sampimon, O. C., Sol, J., Barkema, H. W., Haesebrouck, F., & de Kruif, A. (2004). *In vitro* growth inhibition of major mastitis pathogens by *Staphylococcus chromogenes* originating from teat apices of dairy heifers. *Veterinary Microbiology*, 101(3), 215–221. https://doi.org/10.1016/j.vetmic.2004.03.020

European Union. (2019). Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC (Text with EEA relevance). *Official Journal of the European Union*, L 4, 43–115. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02019R0006-20220128

Stevens, M., Piepers, S., Supré, K., Dewulf, J., De Vliegher, S., & Haesebrouck, F. (2016). Antimicrobial consumption and resistance in bacteria from dairy cattle: A review. *Veterinary Microbiology*, 194, 42–50. https://doi.org/10.1016/j.vetmic.2016.02.008

Toledo-Silva, B., de Souza, F. N., Piepers, S., Mertens, K., Haesebrouck, F., & De Vliegher, S. (2021). Metabolites of bovine-associated non-*aureus* staphylococci influence expression of *Staphylococcus aureus agr*-related genes *in vitro*. *Veterinary Research*, 52, 62.https://doi.org/10.1186/s13567-021-00933-x

Toledo-Silva, B., Beuckelaere, L., De Visscher, A., Geeroms, C., Meyer, E., Piepers, S., Thiry, D., Haesebrouck, F., & De Vliegher, S. (2022). Novel quantitative assay to describe *in vitro* bovine mastitis bacterial pathogen inhibition by non-*aureus* staphylococci. *Pathogens*, 11(2), 264. https://doi.org/10.3390/pathogens11020264

Soundharrajan, I., Yoon, Y. H., Muthusamy, K., Jung, J. S., Lee, H. J., Han, O. K., & Choi, K. C. (2021). Isolation of *Lactococcus lactis* from whole crop rice and determining its probiotic and antimicrobial properties towards gastrointestinal associated bacteria. *Microorganisms*, 9(12), 2513. https://doi.org/10.3390/microorganisms9122513

Cow-Level Bacterial Shedding Is a Dynamic and Herd-Specific Driver of High Bulk Tank Bacterial Counts

Malene Budde[1], Line Svennesen[2], Marie Laurup Petersen[1] Lærke Boye Astrup[1]
Michael Farre[1*]
Seges Innovation, Århus, Denmark[1]
University of Copenhagen, Copenhagen, Denmark[2]
***Corresponding author's email: mifa@seges.dk**

Introduction

Traditionally, elevated bacterial counts in bulk tank milk (BTM) have been associated with issues related to milking equipment hygiene and insufficient milk cooling. Also, management factors such as pre milking sanitation and increased focus on stall management are associated with the BTM bacterial count (Piepers et al., 2014). While these factors remain important, evidence increasingly points towards bacterial shedding from the cows, particularly from cows infected with Streptococcus agalactiae (Group B Streptococci)—as a significant source of cross contamination in many herds (Katholm et al., 2012). Scientific research and field experience have shown that in herds with persistently high Individual Bacteria Counts (IBC), the primary source is often a subset of cows shedding high levels of bacteria, rather than systemic hygiene failures (Hayes et al., 2001; Zadoks et al., 2004).

Despite this, very little research has investigated what bacterial species pose the IBC problem and if they are mastitis pathogens at all. Furthermore, there is no knowledge about the dynamics of IBC, which may be fundamental in decision making.

To address this gap, we focused on herds with elevated BTM bacterial counts identified as infected with S. agalactiae where equipment hygiene and cooling was ruled out as the cause of that. In such cases, individual cows were suspected to be the source of BTM bacterial counts, and the project investigated their role in contaminating the milk.

Understanding the origin of bacterial contamination is essential to ensure milk quality and safety. However, identifying cow-level bacterial shedding is challenging due to variability between herds and variability over time within the same herd. To gain deeper insight into this variation, a pilot project was initiated. These results may contribute to

the development of more targeted and effective strategies for reducing bacterial counts in situations where individual cows are the main source of contamination.

Material & methods

By convenience, a single herd was selected based on a history of fluctuating BTM bacterial counts and persistently variable IBC over a prolonged period. Typically, the IBC fluctuated around 60,000 IBC/mL, with occasionally higher values observed. The geometric mean of the BTM Somatic Cell Count (SCC) was 314,000 cells/mL. The study unit was the individual lactating cow. The herd consisted of 340 Holstein dairy cows, milked two or three times daily, depending on milk yield and stage of lactation.

Prior to sampling, a milk quality advisor assessed the hygiene of the milking system and bulk tank, the dosing of cleaning chemicals, and the cooling system. During the study, all lactating cows had composite milk samples collected. The IBC was measured from these samples, and the procedure was repeated 13 days later. The cows were grouped into four categories according to the measured IBC: < 100.000 IBC/mL, 100.000 – 1. mio. IBC/mL, 1. mio. – 10 mio. IBC/mL, and last > 10 mio. IBC/mL. During sampling, cows were scored for udder hygiene.

All milk samples were cooled and transported to Eurofins® Milk Testing the following day for laboratory analysis.

Following the second sampling, aseptic quarter milk samples were collected from (N=25) cows with the highest IBC values. Microbiological examination was carried out following NMC standards and species identification with MALDI-TOF.

Results & Discussion

The analysis revealed substantial variation in bacterial shedding between individual cows (N=340), with IBC ranging from 3,000 IBC/mL to above 22 million IBC/mL. Most cows (N=312) had relatively low IBC (< 100,000 IBC/mL), but a notable number of cows (N=28) had values above 10 million IBC/m/L.

The repeated sampling showed that bacterial shedding was not consistent over time. Of the (N=28) cows above 10 million IBC/mL in the first sampling, only (N=6) cows remained above 10 million IBC/mL in the second sampling. Seven cows from the same high group dropped below 100,000 IBC/mL. Overall, (N=144) cows, remained in the same IBC/mL category across both test days. This indicates that while some cows are likely persistent high shedders, many cows fluctuate significantly over short periods or are occasionally not cleaned according to the farm protocols.

These findings suggest that elevated bacterial counts in BMT are not always driven by a fixed group of individual high-shedding cows. The variation observed within just 13 days implies that decisions—such as culling— based on a single high IBC may not effectively resolve the issue. Instead, repeated sampling and a more nuanced management approach are needed when addressing elevated IBC.

There was no clear correlation between SCC and IBC if the cow level SCC was below 400,000 cells/mL. Above this threshold, higher SCC often correlated with high IBC (>100.000 IBC/mL). Nearly all cows with high IBC also had high SCC, suggesting that cow level SCC could be used as an indicator of IBC and thereby a useful screening tool.

Another possible contributor to high IBC is udder contamination. However, no relationship was found between hygiene score and IBC, suggesting udder cleanliness was not a good indicator in this case.

Of the (N=25) cows selected for microbiological analysis on quarter level, three cows had all quarters culture negative. Among the culture positives, S. uberis was the most common isolate identified, followed by non-aureus staphylococci and mammaliicoccus (NASM), Corynebacterium spp., Enterococcus cecorum, and S. agalactiae.

These results align with previous findings confirming that Streptococcus spp. and similar pathogens can be associated with shedding in very high numbers. The study highlights the complexity of managing elevated IBC and underscores the need for herd-specific strategies based on repeated testing and pathogen identification.

Conclusion

- High bacterial counts in bulk tank milk can be caused by individual cows, but shedding patterns vary greatly over time. A single test is insufficient to identify persistent high shedders, as shedding appears to fluctuate.
- Cow level SCC may indicate high individual bacterial shedding but is only shown in a single herd.
- Udder hygiene scores did not correlate with individual bacterial shedding in this study.
- Pathogen analysis confirmed *Streptococcus uberis* and NASM as key contributors.

References

Hayes, M. C., Ralyea, R. D., Murphy, S. C., Carey, N. R., Scarlett, J. M., & Boor, K. J. (2001). Identification and Characterization of Elevated Microbial Counts in Bulk Tank Raw Milk. *Journal of Dairy Science*, *84*(1), 292–298. https://doi.org/10.3168/jds.S0022-0302(01)74479-7

Katholm, J., Bennedsgaard, T. W., Koskinen, M. T., & Rattenborg, E. (2012). Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. *Journal of Dairy Science*, *95*(10), 5702–5708. https://doi.org/10.3168/jds.2011-5307

Piepers, S., Zrimšek, P., Passchyn, P., & De Vliegher, S. (2014). Manageable risk factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds. *Journal of Dairy Science*, *97*(6), 3409–3419. https://doi.org/10.3168/jds.2013-7203

Zadoks, R. N., González, R. N., Boor, K. J., & Schukken, Y. H. (2004). Mastitis-Causing Streptococci Are Important Contributors to Bacterial Counts in Raw Bulk Tank Milk. *Journal of Food Protection*, *67*(12), 2644–2650. https://doi.org/10.4315/0362-028X-67.12.2644

Simulation of the outcome of cow and quarter-level mastitis management interventions using test-day somatic cell count data

Pablo Silva Bolona [1*], Natasha Harding[1,2] and Rachael Millar[1,3]

[1] Teagasc, Animal and Grassland Research and Innovation Centre, Fermoy, Co. Cork, Ireland

[2] University College Dublin, Dublin, Ireland

[3] University of Nottingham, Nottingham, United Kingdom

***Corresponding author's email: pablo.silvabolona@teagasc.ie**

Introduction

Mastitis is the most common disease affecting dairy cattle. There have been many management practices suggested and tested for managing mastitis in-lactation or during the dry period, e.g. the 5-point control plan (Neave et al., 1969). However, while many control measures are preventative in nature, few provide immediate changes in cow-level SCC and infection dynamics, resulting in a knowledge gap regarding short-term management strategies.

In Ireland, the majority of subclinical intramammary infections (IMI) are caused by *Staphylococcus aureus* (Clabby et al, 2024), a contagious pathogen that is transmitted to uninfected cows during milking via milkers' hands, cloths, paper towels or the milking cluster. Managing subclinical mastitis during lactation is necessary, particularly in herds where contagious bacteria are the primary source of infection, as such cases can act as a reservoir for transmission within a herd. Some practices for managing mastitis (clinical and subclinical) during lactation have been highlighted, such as segregating high SCC cows to be milked last, drying-off quarters, and antibiotic treatment.

Modelling is a useful tool to assess these impacts. Other modelling approaches have looked at the impact of mastitis control practices (Oostergard et al., 2005) but to our knowledge no model has looked at the impact of interventions based on herd test-day information on SCC in the following test-day. Therefore, the objective of this study was to model the impact of mastitis control interventions based on test-day results on SCC, percentage of chronic high SCC cows and antibiotic treatment.

Material & methods

With data from five commercial dairy herds in Ireland dealing with high bulk tank SCC, we developed a decision algorithm to guide actions (interventions) based on SCC results from milk recordings during lactation.

The suggested intervention involved assessing raw SCC values from milk recordings to determine whether they exceeded or fell below 250,000 cells/ml. Depending on the outcome, the advised actions included: treatment with antibiotics, drying-off the high SCC quarter, doing a CMT in 10 days or do nothing (Figure 1). These are based on other decision trees and recommendations such as that of Pinzon-Sanchez et al. (2011), and Animal Health Ireland (CellCheck, the national mastitis control programme, 2022).

Modelled outcomes

After suggesting interventions, we modelled the impact of these on SCC as follows:

- Antibiotic treatment: If a quarter was treated, we assigned it a 60% chance of cure (van der Borne et al., 2010). Randomly sampling from a uniform distribution, a number between 0 and 1 was drawn and if the value was <0.6 then the cow was deemed "cured". If the draw was >0.6 the cow was deemed "not cured". Cows "cured" would be drawn a SCC value from a uniform distribution between 50,000 cells/mL and 200,000 cells/mL. Cows "not cured" would have been randomly assigned a SCC between 200,000 cells/mL and 5,000,000 cells/mL.
- Drying a quarter: After the quarter was dried, we assumed that the cows SCC would immediately decrease to a value between 50,000 and 200,000 cells/mL (randomly drawn) as a result of not milking that quarter.
- CMT in 10 days: We assumed a 50% chance of spontaneously "curing". If the cow did not cure after 10 days, the cow was assigned to the "antibiotic treatment" intervention and was drawn a SCC value as explained above.
- No action: The SCC assigned to a cow with a "no action" decision was taken from their subsequent milk recording.

The impact of these interventions on cow-level SCC was modelled for the next milk recording on the 5 herds. One hundred iterations of the intervention model were conducted and the average cow level SCC, the percentage of high SCC cows, percentage of dry quarters and percentage of cured infections were estimated. We compared this with the actual following milk recording results.

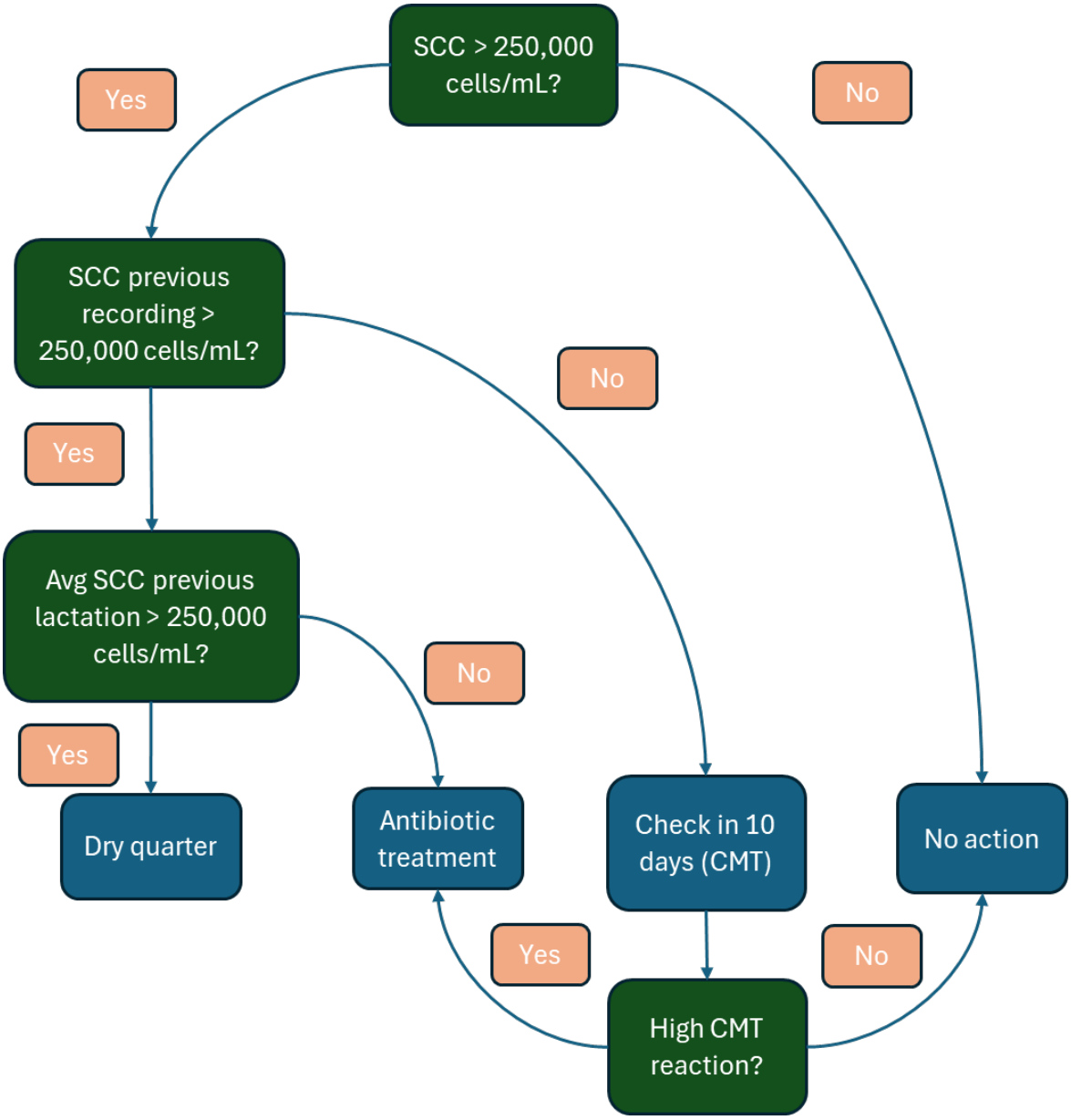


Figure 1. Mastitis interventions decision tree proposed for this study. Green boxes represent questions to guide the decision tree, and blue boxes the suggested interventions.

Average herd size in this study was 167 cows. Of cows that received the interventions (excluding "no action"), persistently high SCC in our model was 49% (range 2 to 8 cows per herd) compared to 79% of cows (average 11 cows per herd) from the actual milk recording. Percentage cure was 51% in our model compared to 21% of the actual milk recording data. With our proposed decision tree, 4% of quarters were dry in the herd compared to 0% in the actual herds. Average cow SCC was 250,000 cells/mL.

Significant challenges remain in managing mastitis during lactation for dairy farmers, including the variable cure rates in-lactation (particularly for mastitis caused by *S. aureus*) and the reluctance of farmers in seasonal calving systems to cull persistently infected cows during lactation. However, the lack of action after milk recording can result in increased spread of infection, increased cases of clinical mastitis and a high

bulk tank SCC. Therefore, it is important to develop simple and targeted management recommendations for farmers to control SCC in the lactation.
A key limitation of this and other simulation models is the validation of their predictions (Oostergard et al., 2005). At this point, we do not have the data of the impact that these interventions have on SCC to validate whether our proposed interventions will achieve the desired outcomes in terms of mastitis control during lactation. Therefore, in future research we will focus on collecting data on the impact of specific interventions to refine the model.

Conclusion

This study showed the mastitis interventions that can potentially contribute to short-term improvement of udder health in dairy herds. By reducing the number of high SCC quarters, farmers can reduce the overall somatic cell count and potentially reduce the rate of new intramammary infections.

References

Animal Health Ireland. 2022. Farm-guidelines for mastitis control. Available at: https://animalhealthireland.ie/programmes/cellcheck/

Clabby, C., Valldecabres, A., Dillon, P., O'Sullivan, K., Arkins, S., Flynn, J., and Silva Boloña, P. 2024. The association between somatic cell count and selective dry cow therapy, milking routine, and dry cow management practices in early-lactation cows from 21 commercial grazing dairy herds. Journal of Dairy Science, 107: 7106-7120.

Neave F.K., Dodd F.H., Kingwell R.G. 1969. Control of mastitis in a dairy herd by hygiene and management. Journal of Dairy Science, 52:696–707. https://doi.org/10.3168/jds.S0022-0302(69)86632-4

Østergaard, S., Chagunda, M. G. G., Friggens, N. C., Bennedsgaard, T. W., and Klaas, I. C. 2005. A stochastic model simulating pathogen-specific mastitis control in a dairy herd. Journal of dairy science, 88: 4243-4257.

Pinzón-Sánchez, C., Cabrera, V. E., and Ruegg, P. L. 2011. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. Journal of Dairy Science, 94: 1873-1892.

van den Borne, B. H., Halasa, T., van Schaik, G., Hogeveen, H., and Nielen, M. 2010. Bioeconomic modeling of lactational antimicrobial treatment of new bovine subclinical intramammary infections caused by contagious pathogens. Journal of Dairy Science, 93: 4034-4044.

Recommendations for Selective Dry Cow Therapy Towards Global Harmonization of Antimicrobial Stewardship in the Dairy Sector

Lærke Boye Astrup[1*] and Michael Farre[1]

[1] Seges Innovation, Århus, Denmark

***Corresponding author's email: lbas@seges.dk**

Introduction

Selective Dry Cow Therapy (SDCT) has become a cornerstone of antimicrobial stewardship in dairy production, particularly following the implementation of EU Regulation (EU) 2019/6, which prohibits prophylactic use of antibiotics in livestock. Since January 2022, this regulation has transformed Dry Cow Therapy across Europe from a preventive, blanket treatment approach to a selective, evidence-based practice. While this represents a major advancement in antimicrobial reduction, it has also highlighted significant gaps in knowledge, data consistency, and practical implementation across the dairy sector.These gaps include: (1) the lack of harmonized definitions of cow- and herd-level eligibility for SDCT; (2) inconsistent use of somatic cell count (SCC) thresholds and diagnostic tools; and (3) the absence of globally accepted protocols integrating management, hygiene, and monitoring standards. The International Dairy Federation (IDF) initiative described here aims to fill these gaps through the development of science-based, globally harmonized recommendations that ensure udder health and milk quality while reducing antibiotic reliance. The work aligns with UN Sustainable Development Goals (SDG 3, 12, and 15) by promoting responsible antimicrobial use, sustainable dairy farming, and improved animal welfare.

Material & methods

The project adopts a structured, evidence-based methodology combining a comprehensive literature review, expert consultation, and comparative gap analysis across existing national and international frameworks. The study is carried out under the IDF Standing Committee on Animal Health and Welfare (SCAHW), and selected global experts in dairy management, veterinary medicine, and policy.

The initial phase of the project involved a systematic gap analysis to map current knowledge and practice discrepancies related to SDCT.

Key steps included:

- Data compilation from peer-reviewed literature, national guidelines, and private assurance schemes.
- Comparative assessment of existing SDCT frameworks to identify variations in eligibility criteria, diagnostic thresholds, antimicrobial decision tools, and risk management procedures.

The gap analysis revealed several critical inconsistencies:

- Divergent SCC thresholds for treatment eligibility (ranging from 100,000 cells/mL to 200,000 cells/mL).
- Incomplete alignment of diagnostic methods (use of CMT, bacteriology, or electronic SCC data).
- Uneven access to veterinary support and data systems across regions.
- Absence of a unified decision-support framework integrating udder health monitoring, antimicrobial stewardship, and economic sustainability.

These gaps underscored the need for a harmonized, evidence-driven approach adaptable to both high-input and emerging dairy systems.

A structured literature review was conducted. Inclusion criteria focused on:

- Randomized or observational studies assessing SDCT efficacy.
- National policy and guideline documents related to antimicrobial use in dairy.
- Meta-analyses evaluating the impact of SDCT on mastitis incidence, SCC, and antimicrobial reduction.

3. Expert Consensus Process

Findings from the gap analysis and literature review will be presented to an IDF Expert Working Group representing academia, veterinary associations, and dairy industry stakeholders. The group develop recommendations across three key dimensions:

1. Eligibility Criteria: Definition of herd-, cow- and quarter-level indicators.
2. Implementation Guidelines: Hygienic procedures, sealant use, monitoring frequency, and data recording.
3. Performance Evaluation: Indicators for post-calving infection rates, antimicrobial usage (DDDA metrics), and herd health outcomes.

The final product will be peer-reviewed and published as an IDF Bulletin, with open access to maximize global uptake.

Results & Discussion

Preliminary findings from the gap analysis show that SDCT, when properly applied, can safely reduce antimicrobial use by 30–70%, maintaining udder health at levels equivalent to or better than blanket therapy. However, implementation success is highly dependent on herd health monitoring infrastructure, farmer training, and data accuracy. Countries with centralized recording systems (e.g., Denmark, the Netherlands) have achieved robust adoption and consistent outcomes, whereas regions lacking structured data collection face challenges in identifying suitable cows for selective therapy. Moreover, the inconsistent definition of "low-risk cows" contributes to confusion and potential udder health risks.

The IDF project's harmonized framework seeks to:

- Establish a risk-based decision tool integrating SCC, infection history, parity, and management factors.
- Promote minimum data standards for eligibility determination.
- Provide best-practice protocols for dry-off hygiene, sealant use, and monitoring.
- Encourage international alignment with NMC and OIE antimicrobial stewardship objectives.

This harmonization will support global comparability of antimicrobial reduction data and ensure that selective approaches maintain high milk quality and animal welfare standards.

Conclusion

The IDF project *"Recommendations for Selective Dry Cow Therapy (SDCT)"* provides a critical step toward global harmonization of antimicrobial use at the dry period. By addressing scientific and practical gaps through systematic analysis and expert consensus, the project will deliver a globally relevant, evidence-based framework for SDCT.The resulting IDF Bulletin will guide dairy professionals, veterinarians, and regulators in adopting selective therapy responsibly balancing antimicrobial reduction with udder health and farm productivity. The recommendations will reinforce the dairy sector's commitment to sustainable farming and responsible medicine use worldwide.

References

European Commission. (2019). *Regulation (EU) 2019/6 on veterinary medicinal products*. Official Journal of the European Union.

Halasa, T., Nielen, M., & Hogeveen, H. (2020). *Selective dry cow therapy: Effects on antimicrobial usage and udder health*. Journal of Dairy Science, 103(12), 11853–11867.

Nielsen, S. S., Bennedsgaard, T. W., & Enevoldsen, C. (2021). *Implementation of selective dry cow therapy in Denmark: Experience and results*. Preventive Veterinary Medicine, 191, 105351.

Ruegg, P. L. (2017). *A 100-year review: Mastitis detection, management, and prevention*. Journal of Dairy Science, 100(12), 10381–10397.

Seasonal impact from management protocols on post calving udder health

Lærke Boye Astrup[1*], Heidi Lund Hyttel[1] and Michael Farre[1]

[1] Seges Innovation, Århus, Denmark

***Corresponding author's email: lbas@seges.dk**

Introduction

Dry cow therapy (DCT) remains a cornerstone in ensuring optimal udder health in dairy cows after calving. Traditionally, blanket dry cow therapy (BDCT) was widely adopted; however, increasing concerns regarding antimicrobial resistance have led to greater emphasis on selective dry cow therapy (SDCT) and evidence-based intervention (Rowe et al., 2023) . While SDCT have shown promise, their efficacy is not uniform throughout the year. Seasonal variations, encompassing fluctuations in temperature and humidity may exacerbate pathogen proliferation and affect cow hygiene, altogether challenging the consistent application and success of SDCT protocols. These seasonal dynamics can lead to variation in the rate of new IMIs during the dry period, impacting both short-term and long-term herd productivity and health. In addition to DCT, internal teat seal can have an additive effect on dry cow infection status (McArt & Wieland, 2024).Therefore, a comprehensive understanding of how seasonality influences the biological, environmental, and management factors surrounding dry cow therapy is essential. This understanding is particularly critical in the context of antimicrobial stewardship and precision livestock farming, where tailored interventions are increasingly required. All this is often compensated for in scientific literature, but in on-farm consulting such contexting is often not addressed when evaluating the protocols applied on the farm.

Material & methods

Data were obtained from Danish dairy herds enrolled in official milk recording (DHI) between 2022 and 2024. Only DHI herds were included, as somatic cell count (SCC) data – essential for identifying new intramammary infections – are only available for these herds. In the analysis we have included (N= 957.282) observations, from (N=576.232) cows and (N=1.746 herds). Only cows that had a milk recording with

somatic cell count (SCC) within 100 days before drying off and another milk recording with SCC within 100 days after calving were included in the analysis.
Cows were classified into four mutually exclusive groups based on dry-off treatment records from the Danish national database:

1. Antibiotic dry cow therapy (DCT)
2. Internal teat sealant (ITS)
3. Combination therapy (antibiotics + ITS)
4. No registered dry-off treatment

Re-treatments were not analyzed separately, as they were assumed to be infrequent and unlikely to influence the overall results. For cows without a registered dry-off date, the dry-off was estimated retrospectively by subtracting a fixed number of days from the recorded calving date. This number was determined by calculating the median dry period length from cows with both dry-off and calving dates available. The median was used as a robust estimate for consistent application across groups.

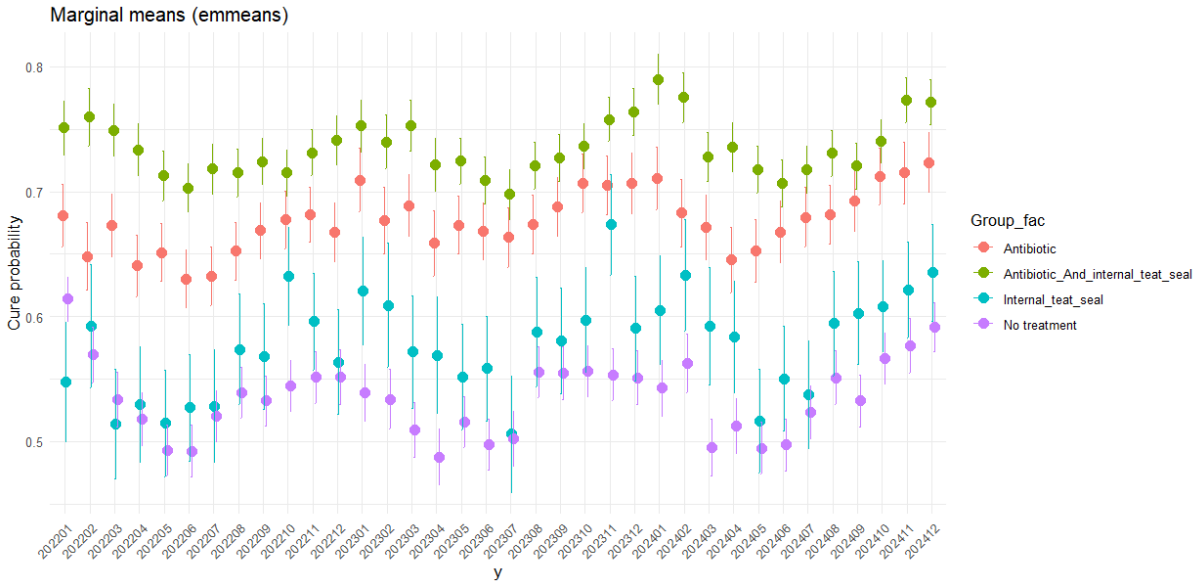
The new intramammary infection rate during the dry period was calculated following the International Dairy Federation (IDF) definition. A cow was defined as newly infected if:

- The last SCC before dry-off was ≤ 200,000 cells/mL, and
- The first SCC after calving was > 200,000 cells/mL.

Only cows with valid SCC records in milk recording were included. The rate was expressed as the proportion of cows that were negative prior to dry-off and became positive after calving. Culling was defined only as slaughter; cows sold for live export or breeding were excluded due to data complexity and differing management intentions. Culling data was used to assess the relationship between treatment group and potential failure to cure. A defined post-calving observation window for culling was not applied.

Results & Discussion

The graph shows cure rates (%) across different treatment groups during the dry period, measured monthly from January 2022 to December 2024. Combination therapy, the green line, consistently achieved the highest cure rates, ranging between 74% and 75% throughout the study period. Cure rates remained stable with minimal fluctuations, indicating the most effective and reliable treatment strategy.
Antibiotic Only, the red lined, showed cure rates between 66% and 70%, with slightly more variability over time. While effective, this treatment was less successful than the combination therapy. Internal Teat Seal Only, the blueline, showed cure rates from 55% to 58%, with considerable variation and moderate fluctuations. This group performed considerable worse than both antibiotic-treated groups, indicating reduced efficacy when used alone.No Treatment, the purple line, showed the lowest cure rates, typically between 51 % and 52%, with moderate month-to-month variation. These results suggest limited spontaneous recovery without any intervention.
While minor seasonal fluctuations were observed, the combination of antibiotics and teat sealant consistently outperformed all other treatments.



The analysis of the seasonal variation is ongoing and will be presented at the conference in March 2026.

Conclusion

- Combination treatment with antibiotics and internal teat seal is the most effective approach during the dry period.
- Antibiotics alone are moderately effective but less than the combination.
- Internal teat seal alone and no treatment result in substantially lower cure rates, highlighting the importance of active intervention.

References

McArt, J. A. A., & Wieland, M. (2024). Efficacy of internal and external teat sealants on cure and new infection risk in dry-off protocols for Holstein cows. *JDS Communications*, *5*(6), 644–648. https://doi.org/10.3168/jdsc.2024-0574

Rowe, S., Kabera, F., Dufour, S., Godden, S., Roy, J.-P., & Nydam, D. (2023). Selective dry-cow therapy can be implemented successfully in cows of all milk production levels. *Journal of Dairy Science*, *106*(3), 1953–1967. https://doi.org/10.3168/jds.2022-22547

Metabolites of bovine-related non-*aureus* staphylococci and mammaliicocci modulate *Staphylococcus aureus* virulence and epithelial immune response *in vitro*

Gregory Rota[1]*, Ana C. D. Oliveira[1], Silvia Beschi[1], Kristien Mertens[1], Bruno Toledo-Silva[1], Sofie Piepers[1], Fernando N. Souza[1, 2], Damien Thiry[3], Evelyne Meyer[1] and Sarne De Vliegher[1]

[1] Ghent University, Merelbeke, Belgium
[2] Universidade de São Paulo, São Paulo, Brazil
[3] University of Liège, Liège, Belgium

***Corresponding author's email: gregory.rota@ugent.be**

Introduction

Bovine mastitis remains the most economically significant disease in the dairy industry, causing substantial financial losses and welfare concerns (Ruegg, 2017). It is primarily caused by bacterial pathogens, with *Staphylococcus aureus* (SA) being one of the most clinically significant due to its high virulence and ability to internalize into bovine mammary epithelial cells (bMECs), facilitating chronic intramammary infection (IMI) and immune evasion (Cobirka et al., 2020; Toledo-Silva et al., 2023). Despite global efforts to reduce antibiotic use, mastitis remains the leading cause of antibiotic administration in dairy farming, underscoring the need for non-antibiotic alternatives (Stevens et al., 2016).

In this context, specific non-*aureus* staphylococci and mammaliicocci (NASM) isolates have emerged as potential protective agents of udder health. This study aimed to characterize NASM-derived metabolites and investigate whether cell-free supernatants (CFS) from four bovine-associated NASM isolates, previously selected for their inhibitory properties, affect SA adherence, internalization, and the immune response of bMECs.

Material & methods

CFS-preparation: Four bovine-associated NASM isolates [*S. chromogenes* TA (SCTA), from a teat apex (De Vliegher et al., 2004); *S. chromogenes* IM (SCIM), from an IMI (Supré et al., 2011); *S. simulans* SS1, from IMI; and *S. simulans* SS10, from a teat apex (Toledo-Silva et al., 2023)] were cultured and their CFS were obtained as previously (Toledo-Silva et al., 2023). The SA 8325-4 strain (Novick and Morse, 1967) was used as the major pathogen to challenge MAC-T cells.

SA treatment: *SA* cultures (2 x 10^4 CFU/mL) were washed and resuspended in the different NASM CFS. These cultures (NASM CFS + SA; n = 4 plus non-treated controls) were allowed to interact for 2 h prior to challenging MAC-T cells. NASM CFS aliquots collected before and 2h after treatment with CFS were analysed for metabolite levels by liquid chromatography-mass spectrometry.

Cell culture: MAC-T cells were cultured in appropriate media at 37°C with 5% CO_2, following established protocols (Souza et al., 2016). The MAC-T cells were challenged with treated or untreated *SA* [multiplicity of Infection (MOI) 1:1]

Adherence and internalization assays: Adherent and internalized bacteria were quantified at 1 and 3h after challenging with treated or untreated SA following standard protocols (Toledo-Silva et al., 2023).

Immune response ELISA assessment: MAC-T supernatants were collected at 0, 1, 3, 6, and 12 h post-challenge with treated or untreated SA. TNF-α, GM-CFS and β-defensin 2 concentrations were quantified by ELISA following manufacturer protocols.

Results & Discussion

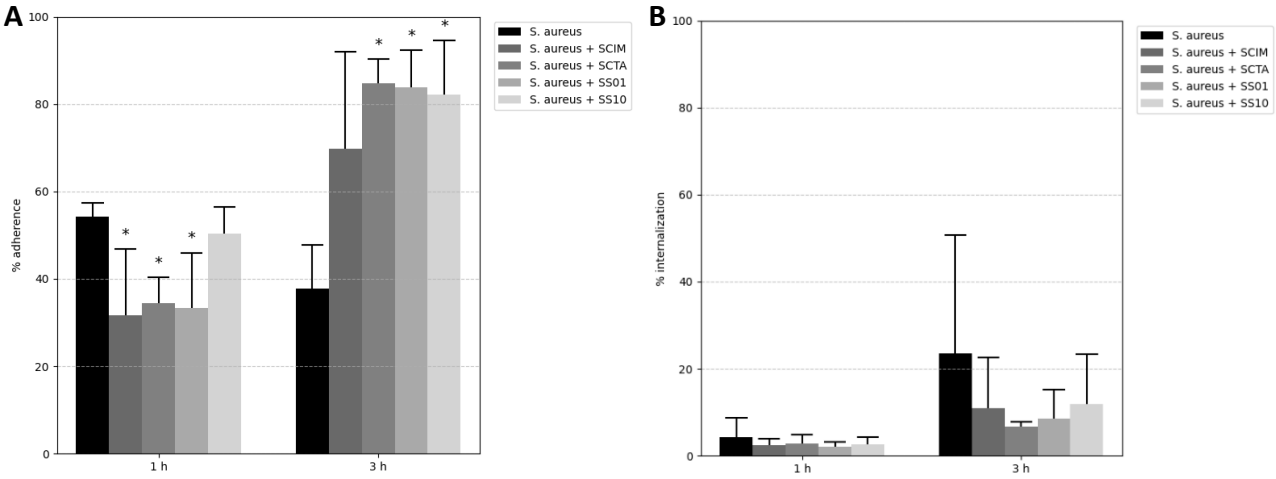


Figure 1 (A) Adherence (%) of SA relative to total count (mean ± SD, n = 3). (B) Internalization (%) of intracellular SA relative to total count (mean ± SD, n = 3).

Adherence and internalization: Prior contact (=treatment) with NASM CFS (group) reduced the ability of SA to adhere to MAC-T cells already at early stages (Figure 1A). Despite variability, NASM metabolites initially delay bacterial attachment but subsequently promote sustained surface association, maintaining bacteria adhered to the cell surface while limiting their internalization (Figure 1B). Notably, SCTA most effectively inhibited internalization, suggesting that NASM metabolites prevent intracellular entry and enhance pathogen exposure to immune surveillance.

Immune response assays: NASM CFS modulate the inflammatory profile of MAC-T cells during SA challenge, showing an early anti-inflammatory effect followed by a delayed

proinflammatory activation. At 6 h, untreated SA induced approximately 0.054 ng/mL TNF-α, whereas NASM pre-treatments reduced secretion to as low as 0.012 ± 0.010 ng/mL. By 12 h, TNF-α levels rose again, reaching up to 0.065 ± 0.006 ng/mL with SS10, indicating that NASM CFS do not block inflammation entirely but rather delay and enhance the response, potentially promoting a more controlled immune activation at later stages of infection. Similarly, prior contact with NASM CFS enhanced β-defensin 2 secretion, increasing from 137 ± 25 pg/mL in MAC-T cells infected with untreated SA to nearly 2916 ± 866 pg/mL with SS10 at 12 h, highlighting an enhanced epithelial antimicrobial stimulation of this NASM isolate.

Metabolite characterization and GM-CSF quantification are currently ongoing to identify the specific bioactive compounds related for these regulatory effects and to further elucidate their contribution to immune modulation during SA infection.

Conclusion

In summary, metabolites from bovine NASM isolates attenuate SA virulence and modulate its interaction with epithelial cells *in vitro*, leading to reduced invasion and a more balanced immune response. These findings highlight the potential of NASM metabolites as modulators of pathogen behaviour and host–pathogen dynamics.

References

Cobirka, M., Tancin, V., & Slama, P. (2020). Epidemiology and classification of mastitis. *Animals*, 10, 2212.

De Vliegher, S., Opsomer, G., Vanrolleghem, A., Devriese, L., Sampimon, O., Sol, J., Barkema, H. W., Haesebrouck, F., & de Kruif, A. (2004). *In vitro* growth inhibition of major mastitis pathogens by *Staphylococcus chromogenes* originating from teat apices of dairy heifers. *Veterinary Microbiology*, 101, 215-221.

Novick, R., & Morse, S. I. (1967). *In vivo* transmission of drug resistance factors between strains of *Staphylococcus aureus*. *Journal of Experimental Medicine*, 125, 45-59.

Piepers, S., Opsomer, G., Barkema, H. W., de Kruif, A., & De Vliegher, S. (2010). Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and higher milk production in their first lactation than noninfected heifers. *Journal of Dairy Science*, 93, 2014-2024.

Piepers, S., Schukken, Y., Passchyn, P., & De Vliegher, S. (2013). The effect of intramammary infection with coagulase-negative staphylococci in early lactating heifers on milk yield throughout first lactation revisited. *Journal of Dairy Science*, 96, 5095-5105.

Ruegg, P. L. (2017). A 100-year review: Mastitis detection, management, and prevention. *Journal of Dairy Science*, 100, 10381-10397.

Souza, F. N., Piepers, S., Della Libera, A., Heinemann, M. B., Cerqueira, M., & De Vliegher, S. (2016). Interaction between bovine-associated coagulase-negative staphylococci species and strains and bovine mammary epithelial cells reflects differences in ecology and epidemiological behavior. *Journal of Dairy Science*, 99, 2867-2874.

Souza, F., Santos, K., Ferronatto, J., Ramos Sanchez, E., Toledo-Silva, B., Heinemann, M. B., De Vliegher, S., & Della Libera, A. (2023). Bovine-associated staphylococci and mammaliicocci trigger T-lymphocyte proliferative response and cytokine production differently. *Journal of Dairy Science*, 106, 2772-2783.

Stevens, M., Piepers, S., Supré, K., Dewulf, J., & De Vliegher, S. (2016). Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and

associations with udder health, milk quality, and production performance. *Journal of Dairy Science*, 99, 2118-2130.
Supré, K., Haesebrouck, F., Zadoks, N., Vaneechoutte, M., Piepers, S., & De Vliegher, S. (2011). Some coagulase-negative *Staphylococcus* species affect udder health more than others. *Journal of Dairy Science*, 94, 2329-2340.
Toledo-Silva, B., de Souza, F., Piepers, S., Mertens, K., Haesebrouck, F., & De Vliegher, S. (2021). Metabolites of bovine-associated non-aureus staphylococci influence expression of *Staphylococcus aureus* agr-related genes *in vitro*. *Veterinary Research*, 52, 62.
Toledo-Silva, B., Oliveira, A., Souza, F., Haesebrouck, F., & De Vliegher, S. (2023). Metabolites of non-aureus staphylococci affect the ability of *Staphylococcus aureus* to adhere to and internalize into bovine mammary epithelial cells. *Veterinary Research*, 54, 100.

Quarter-based vs cow-based selective dry cow therapy

Nadja Alsted [1*], Carsten Kirkeby[1], Volker Krömker[1,2], Leonardo V. de Knegt[1], Lærke B. Astrup[3], Michael Farre[3], Pauline Katthöfer[2], Franziska Nankemann[2], Julia Nitz[2] and Line Svennesen[1]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] Hannover University of Applied Sciences and Arts, Hannover, Germany
[3] SEGES Innovation P/S, Aarhus, Denmark
***Corresponding author's email: nadja.alsted@sund.ku.dk**

Introduction

Mastitis is one of the biggest global health challenges for adult dairy cattle. The dry period is a high-risk period for new infections that can later develop into clinical mastitis (CM), or elevated somatic cell counts (SCC) during the following lactation. Therefore, dry cow therapy (DCT) is used to eliminate existing infections and prevent new intramammary infections (NIMI) during the dry period (Halasa et al., 2009; Ruegg, 2017). Due to emerging antimicrobial resistance, there is a need to reduce the antimicrobial usage (AMU) (ECDC et al., 2024). Therefore, it is no longer allowed to use blanket DCT in the EU (European parliament, 2022). As an alternative, there are two ways to perform selective DCT (SDCT): cow-based (C-SDCT) and quarter-based (Q-SDCT). For C-SDCT, earlier CM cases and/or somatic cell count (SCC) level determine which cows are selected for treatment in all 4 quarters. For Q-SDCT, quarters are selected for treatment by, for example, CMT or bacterial culture (Dufour et al., 2019).
The objective of this study was to compare udder health outcomes and AMU between Q-SDCT and C-SDCT.

Material & methods

The study period was from fall 2022 to the beginning of 2024. Based on convenience and farmers' willingness to participate, we enrolled farms fulfilling the following criteria: conventional, >200 cows, free from *Salmonella Dublin*, milking parlor and enrolled in the Dairy Herd Improvement (DHI) system. Cows were included if they had an SCC above 200,000 cells/mL in the last DHI prior to sampling, had 4 functional quarters and were not treated with antimicrobials 1 month prior to sampling. Quarter milk samples were aseptically collected 1-4 weeks before dry-off and 3-11 days post-calving. All quarter

samples were analyzed with bacterial culture and MALDI-TOF for species identification, and SCC counts were recorded.
Cows were randomly allocated to the C-SDCT or Q-SDCT treatment groups based on their ear-tag number (odd or even). In the C-SDCT group, all quarters of all cows were treated with antimicrobials at dry-off, while only quarters with detected pathogen(s) were treated with antimicrobials in the Q-SDCT group. The treatment was carried out by the farmer and besides the treatment strategy, dry cow management was performed as usual in the study farms.
Bacteriological cure rate, NIMI rate, CM risk and AMU were compared between Q-SDCT and C-SDCT in an initial analysis. Multivariable analyses will be carried out, considering herd, cow and quarter-specific information such as parity, detected pathogen(s) and the use of internal teat sealant (ITS).

Results

We present some crude estimates as preliminary results here.
Eight farms were enrolled in the study. The farms performed different management practices related to dry-off, in example 7 out of 8 farms used ITS for all quarters.
In total, 279 cows (N=1,116 quarters) were dried off, with 588 quarters in the C-SDCT group and 528 quarters in the Q-SDCT group. After exclusion of cows receiving the wrong treatment, the AMU was calculated based on 892 quarters. In the C-SDCT group, 100% of quarters were treated, whereas only 57.2% of quarters were treated in the Q-SDCT group.
After exclusion of cows treated with antimicrobials other than the dry-off treatment between dry-off and post-calving sampling, the analysis of CM cases was based on 205 cows with available treatment data for the dry period and first 30 days in milk. For Q-SDCT, 9.9% of the cows had at least one treated CM, whereas it was 8.1% in the C-SDCT group.
The NIMI rates were 18.3% and 8.9% for Q-SDCT and C-SDCT, respectively. This analysis was based on 661 quarters, infected and non-infected at dry-off.
Bacteriological cure rates were 97.0% and 94.7% for Q-SDCT and C-SDCT, respectively. This analysis was based on 360 quarters infected at dry-off.

Discussion

In Denmark, it is allowed to dry off with antimicrobials if SCC is above 200,000 cells/mL in 2 DHI tests (4 months period) before dry-off. Otherwise, a pathogen should be detected in the milk by PCR or bacterial culture (Ministry of Food, Agriculture and Fisheries of Denmark, 2025). Thereby, the C-SDCT approach may be performed based on inflammation level (SCC) of composite samples alone, leading to treatment of cows with unknown infection status. The preliminary results of this study support the expected reduction in number of treated quarters if Q-SDCT is performed as an alternative to C-SDCT. However, an issue of transitioning to Q-SDCT would likely be an increased amount of labor and cost for quarter-level sampling and testing, as well as processing the microbiological findings into a specific treatment protocol.

Conclusion

The conclusions for this study will be developed following the final results, according to whether the AMU reduction and the transition from C-SDCT to Q-SDCT will result in decreased udder health or not.

References

Dufour, S., Wellemans, V., Roy, J. P., Lacasse, P., Ordonez-Iturriaga, A., & Francoz, D. (2019). Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. *Animal Health Research Reviews*, *20*(1), 86–97.

ECDC, EFSA, & EMA. (2024, February). *Fourth joint inter-agency report on integrated analysis of antimicrobial consumption and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the European Union (JIACRA IV-2019-2021)*.

European parliament. (2022, January) *Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on Veterinary Medicinal Products and Repealing Directive 2001/82/EC (2022)*.

Halasa, T., Nielen, M., Whist, A. C., & Østerås, O. (2009). Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. *Journal of Dairy Science*, *92*(7), 3150–3157.

Ministry of Food, Agriculture and Fisheries of Denmark. (2025, October) *Bekendtgørelse om dyrlægers anvendelse, udlevering og ordinering m.v. af lægemidler til dyr (BEK nr. 1186)*.

Ruegg, P. L. (2017). A 100-Year Review: Mastitis detection, management, and prevention. *Journal of Dairy Science*, *100*(12), 10381–10397.

Estimation of mastitis cases throughout a year, based on quarter milk sample submission and pathogen identification

Rachael Millar [1,2*], Jake Thompson[2], Della Della[1], Luke O'Grady[3], Martin Green[2], Chris Hudson[2], and Pablo Silva Bolona[1]

[1] Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 C302, Ireland

[2] University of Nottingham, Sutton Bonington, Loughborough, United Kingdom.

[3] University College Dublin, Belfield, Dublin 4, Ireland.

***Corresponding author: rachael.millar@teagasc.ie**

Introduction

Mastitis, an inflammation of the mammary gland, is one of the most significant diseases affecting dairy cattle worldwide. It's commonly classified as clinical mastitis (CM) or subclinical mastitis (SCM). CM is expressed as observable signs of inflammation and changes in the milk. SCM has no visible signs but there's an inflammatory process occurring (Harmon, 1994; Keane et al., 2013).

A study carried out by Reksen et al. (2006) in Norway found *Staphylococcus aureus* the most prevalent bacteria found in CM samples from 2,492 randomly sampled cows. Similarly, Keane et al. (2013) found that *Staphylococcus aureus* was the prevalent (23%) pathogen causing CM, followed by *Streptococcus uberis* (17%) and *Escherichia Coli* (9%) in high SCC herds in Ireland. In Ireland, there are few studies examining the epidemiology of CM in dairy herds.

This highlights the need for updated research reflecting current CM levels and pathogen profiles. Therefore, the aim of this study was to estimate the incidence rate and identify the causative bacteria of CM on Irish dairy farms.

Material & methods

This study was granted ethical approval by The University of Nottingham, School of Veterinary Medicine and Science Ethical Review Committee and the Teagasc Ethical Committee.

We collected data from 83 commercial dairy farms for a 1-year period (April 2024-May 2025). The farms were randomly selected from a list of respondents from a survey previously conducted in Teagasc (Uí Chearbhaill et al., 2024). The number of farms selected to participate was proportionally sampled by province based on the National Farm Survey in 2022. The survey recorded that 72% of dairy farms in Ireland are located in Munster, 14% in Leinster and 14% in Connacht and Ulster combined.
Each participating farm was visited three times during the study period. An assessment of farm facilities, milking management and animals, along with a questionnaire was carried out during each visit. Each farmer provided mastitis treatment records and milk recording data. During the initial farm visit, farmers were trained to collect aseptic quarter milk sampling. Farmers were requested to sample all CM detected throughout the study period, before treatment was administered. Samples were frozen on-farm immediately.
Samples were transported frozen to the laboratory for analysis. CM samples were thawed and 10 µL of milk were plated on blood agar. Plates were evaluated based on colony morphology and haemolytic activity after 24 and 48 hours of incubation. Samples with 1 or 2 distinct colony types, with each type containing more than five colonies, were further tested on semi-selective and selective agars respectively to identify presumptive pathogens.
Descriptive statistics of herd size and management practices were calculated. Additionally, we calculated mastitis incidence based upon CM milk samples submitted by farmers. The calculation was, total cases submitted from all farms divided by the total cow numbers on all farms multiplied by 100.

Results

The average herd size in this study was 127 cows (range: 46 to 455). Within the study population, 28% of farms carried out blanket dry cow therapy and 72% carried out selective dry cow therapy. Fifty-five percent of farms calved the cows in grouped pens while 45% calved in single pens.

The median CM incidence was 6.5 cases per 100 cows per year (min: 0, mean: 8.4, max: 27.4). There was seasonal variation in CM case submissions (Figure 1). From April 2024, CM cases submitted were high, peaking in June (>70). From July onwards, a steady decline in CM is clear, reaching a nadir in December. Cases began to increase from January to >30 cases submitted in February.

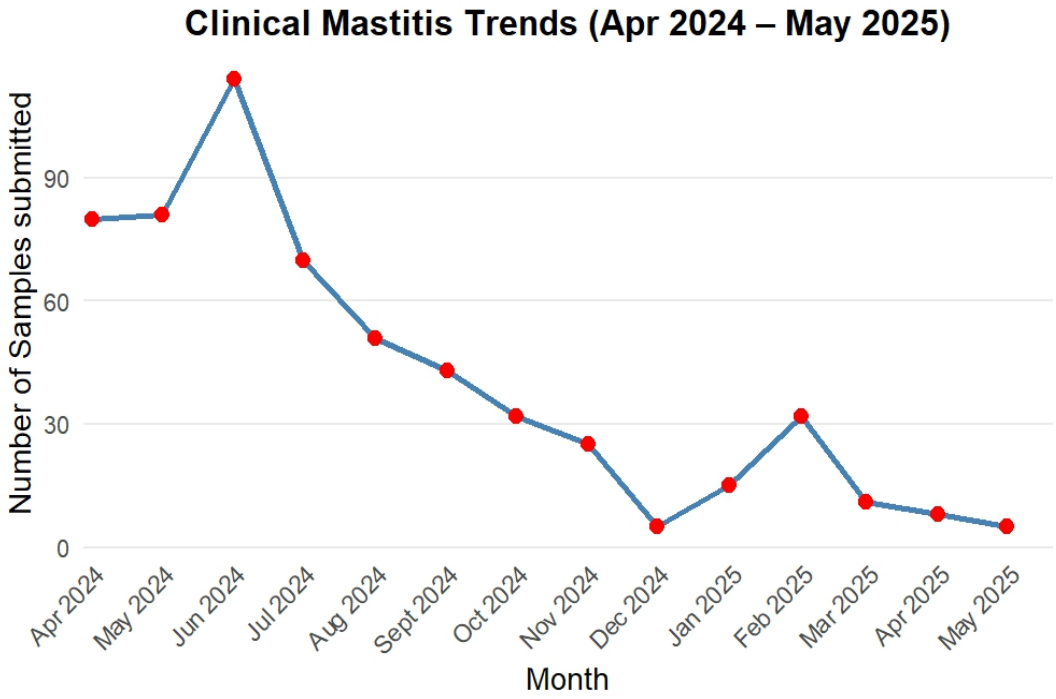


Figure 2: Clinical mastitis trends

Of the 83 herds enrolled in the study, 77 (92.8%) submitted CM samples (range: 1-63 samples). We tested 853 CM samples to identify bacterial presence. Preliminary pathogen identification revealed *Streptococcus* species as the predominant pathogen (18.8%). This was followed by *Escherichia coli* (14%) and *Staphylococcus aureus* (5.9%). Species that were unable to be identified accounted for 18.4% of total sample submission.

Discussion

The CM incidence is likely to be an underestimation as some farmers may have failed to collect samples from all cases, resulting in incomplete case occurrence in this calculation. Further exploration of this will be carried out alongside CM treatment records to establish a more accurate incidence of CM.

Most participating herds (73 of 83) operated a spring-calving system, nine operated split-calving and one calved year-round. This seasonal pattern explains the decline in CM cases during December, when majority of cows were dried off. Additionally, the subsequent rise from January onwards coincides with the peak calving period for 88% of farms. These results suggest the influence of seasonal calving and management practices on CM patterns.

The majority of the bacteria identified seems to be from environmental sources (such as coliform bacteria and *Streptococcus spp.)* which indicates a need for farmers to improve cleanliness level and management of facilities, especially near parturition. The presence of *Staphylococcus aureus* also indicates a need to deal with contagious transmission and with chronically infected cows in some herds. Further analysis (MALDI-TOF) will be carried out to uunidentified samples and *Streptococcus* species to determine bacterial species.

Future risk factor analysis will identify specific management practices associated with CM incidence. Further analysis will be carried out using the stabilizer triangulation modelling method to discover variables and key risk factors given the wide dataset obtained.

Conclusion

Preliminary results show *Streptococcus* species as the predominant species identified. The seasonal variations of CM occurrence highlight the importance of exploring the risk factors linked to peak timepoints. Further research will explore current incidence rate and factors associated with CM specific to the Irish context.

References

Bannerman, D. D. (2009). Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows1. *Journal of Animal Science*, *87*(suppl_13), 10-25. https://doi.org/10.2527/jas.2008-1187

Bianchi, R. M., Schwertz, C. I., de Cecco, B. S., Panziera, W., De Lorenzo, C., Heck, L. C., Snel, G. G., Lopes, B. C., da Silva, F. S., & Pavarini, S. P. (2019). Pathological and microbiological characterization of mastitis in dairy cows. *Tropical animal health and production*, *51*(7), 2057-2066.

Harmon, R. (1994). Physiology of mastitis and factors affecting somatic cell counts. *Journal of Dairy Science*, *77*(7), 2103-2112.

Keane, O. M., Budd, K. E., Flynn, J., & McCoy, F. (2013). Pathogen profile of clinical mastitis in Irish milk-recording herds reveals a complex aetiology. *Veterinary Record*, *173*(1), 17-17. https://doi.org/https://doi.org/10.1136/vr.101308

Reksen, O., Sølverød, L., Branscum, A., & Østerås, O. (2006). Relationships between milk culture results and treatment for clinical mastitis or culling in Norwegian dairy cattle. *Journal of Dairy Science*, *89*(8), 2928-2937.

Uí Chearbhaill, A., Boloña, P. S., Ryan, E. G., McAloon, C. I., Burrell, A., McAloon, C. G., & Upton, J. (2024). Survey of farm, parlour and milking management, parlour technologies, SCC control strategies and farmer demographics on Irish dairy farms. *Irish veterinary journal*, *77*(1), 8.

Decoding bacterial dynamics in bovine mastitis: a proteomic exploration of *S. aureus* and Non–Aureus Staphylococci and Mammaliicocci in milk

Alicja Krysmann [1], Morten Kjos[1] and Davide Porcellato[1]

1- Norwegian University of Life Sciences, Aas, Norway

***Corresponding author's email: alicja.monika.krysmann@nmbu.no**

Introduction

Mastitis, an inflammatory mammary gland disease, is a significant challenge in the dairy industry due to its economic impact and animal welfare implications. Among the wide variety of microorganisms that can be a cause of mastitis, *Staphylococcus aureus* is recognized as one of the major pathogens responsible for contagious mastitis. It can be transmitted during milking and can easily spread within the herd. The problematic nature of *S. aureus* is related to its ability to persist in the mammary gland and the difficulty of antibiotic treatment, as mastitis-causing strains are often highly adapted to the udder environment (Campos et al., 2022; Grunert et al., 2018). In contrast, the role of non–aureus staphylococci and mammaliicocci (NASM) in bovine mastitis is less well understood (Crippa et al., 2024). Although NASM are among the most frequently isolated bacteria from milk, they are rarely associated with clinical mastitis (Hamel et al., 2020).

By sequencing methods of hindmilk at the quarter level, we previously detected the presence of *S. aureus* with NASM in the same quarter. To date, however, only a limited number of studies have explored the interplay between *S. aureus* and NASM.

Some of the NASM species, such as *Staphylococcus epidermidis* have been previously detected together with *S. aureus*, whereas others, like *Staphylococcus chromogenes* were not associated with *S. aureus*. This pattern suggests a complex set of interactions and species-specific behavior. However, there is a limited number of studies exploring these possible interactions among members of the udder microbiome. In this study, we

investigated the *S. aureus* proteomic profile in coculture with selected NASM strains isolated from milk to gain insights into its activity and potential interspecies interactions.

Material & methods

Bacterial strains (*S. aureus, S. chromogenes* and *S. epidermidis*) isolated from bovine hindmilk were cultivated in milk at 37°C alone or in coculture and their proteome was analyzed after 8 and 72 hours of incubation. The expressed proteins were analyzed by LC-MS/MS with a gel-free suspension trapping sample preparation method (Zougman et al., 2014). Raw data were processed with Max-Quant, and differential abundance between the expressed proteins was analyzed for *S. aureus* alone or in coculture with the other two strains.

Results & Discussion

There were total of 925 *S. aureus* proteins detected in the samples. Differential enrichment analysis showed some significant changes in the proteome of SA grown in monoculture compared to co-culture with *S. chromogenes*. The presence of *S. chromogenes* affected the expression of nearly 30 proteins (3% change in expression), with 12 proteins upregulated and 17 downregulated during co-culture. After functional analysis, the overexpressed proteins in co-culture were found to be involved in stress response pathways, including superoxide dismutase and oxygen-dependent choline dehydrogenase. In co-culture with *S. epidermidis,* only 10 proteins in *S. aureus* were altered, including upregulation of thioredoxin. These proteins are also linked to stress response of *S. aureus* and they are responsible for reducing reactive oxygen species as a response to oxidative stress and general adaptation to changing environment.

Conclusion

The results show that *S. aureus* is negatively affected by the presence of NASM, particularly *S. chromogenes,* in the milk. While the exact role of NASM in mastitis is not clear, our results indicate that they might have a potential role in regulating microbiome dynamics in the udder and interact with pathogens to modulate their physiology.

References

Campos, B., Pickering, A. C., Rocha, L. S., Aguilar, A. P., Fabres-Klein, M. H., de Oliveira Mendes, T. A., Fitzgerald, J. R., & de Oliveira Barros Ribon, A. (2022). Diversity and pathogenesis of Staphylococcus aureus from bovine mastitis: Current understanding and future perspectives. *BMC Veterinary Research*, *18*, 115. https://doi.org/10.1186/s12917-022-03197-5

Crippa, B. L., Matos, L. G. de, Souza, F. N., & Silva, N. C. C. (2024). Non-aureus staphylococci and mammaliicocci (NASM): Their role in bovine mastitis and One Health. *Journal of Dairy Research*, *91*(1), 44–56. https://doi.org/10.1017/S0022029924000165

Grunert, T., Stessl, B., Wolf, F., Sordelli, D. O., Buzzola, F. R., & Ehling-Schulz, M. (2018). Distinct phenotypic traits of Staphylococcus aureus are associated with persistent, contagious bovine intramammary infections. *Scientific Reports*, *8*(1), 15968. https://doi.org/10.1038/s41598-018-34371-1

Hamel, J., Zhang, Y., Wente, N., & Krömker, V. (2020). Non-*S. aureus* staphylococci (NAS) in milk samples: Infection or contamination? *Veterinary Microbiology*, *242*, 108594. https://doi.org/10.1016/j.vetmic.2020.108594

Zougman, A., Selby, P. J., & Banks, R. E. (2014). Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis. *PROTEOMICS*, *14*(9), 1006–1000. https://doi.org/10.1002/pmic.201300553

Associations between *Streptococcus uberis* mastitis and herd characteristics

Muusa Takala [1], Päivi Rajala-Schultz[1], Tiina Autio[2], Riitta Niemi[1*]

[1] University of Helsinki, Mäntsälä, Finland

[2] Finnish Food Authority, Kuopio, Finland

***Corresponding author's email: muusa.takala@helsinki.fi**

Introduction

Streptococcus uberis is a common mastitis pathogen among dairy cows, capable of causing both environmental and contagious infections (Klaas & Zadoks, 2018). While it is a common finding both in cow and its environment, transmission route and environmental hotspots seem to be both strain and herd specific (Wente et al., 2019). The genetic diversity of *Str. uberis* within a herd is linked to differences in farm management practices (Srithanasuwan et al., 2025). However, there is limited information on herd-specific differences in the proportional morbidity (PM) of *Str. uberis* intramammary infection (IMI) and on how these differences are associated with herd characteristics. This study aimed to assess PM of *Str. uberis* IMI across dairy herds and to investigate the associations between herd characteristics and increased PM of *Str. uberis* mastitis across time.

Material & methods

This retrospective observational study utilized the Finnish Dairy Herd Improvement (DHI) records from the national milk recording database (MTech Digital Solutions, Finland) and quarter-level milk sample results from the Voluntary Centralized Cattle Health Care Register (Animal Health ETT), for the years 2013, 2018, and 2023. Data comprised 361 023 quarter-level milk samples, and DHI records from 14 424 herds. Milk samples were collected for suspected clinical or subclinical mastitis and analyzed using quantitative real-time PCR (qPCR) with Thermo Scientific PathoProof Mastitis Complete-16 assay (Thermo Fisher Scientific Ltd., Vantaa, Finland). Based on the qPCR results, the samples were classified as IMI-positive, IMI-negative, or contaminated. Annual national-level *Str. uberis* PMs were calculated as the proportion of *Str. uberis* IMI-positive samples to the total of IMI-positive samples for each year. The primary outcome of the statistical model was the annual herd-specific PM for *Str. uberis*, calculated as the number of *Str. uberis* IMI-positive quarter samples divided by the total number of IMI-positive samples for each herd. Bootstrapping was used to increase the statistical power of the outcome

variable. These bootstrapped *Str. uberis* PM estimates were classified as 'high' or 'normal/low' if both their upper and lower bound of 95% confidence interval (CI) were above or below the set threshold. The threshold between the categories was 19%, based on the national-level annual PM. If 95% CI included the threshold value, the estimate was considered not classifiable. Herds that met the inclusion criteria and were successfully classified were selected for further analysis. To avoid clustering, the statistical model included one observation per herd, prioritizing the most recent observation over earlier ones, resulting in a sample of 1 839 herds. A Bernoulli distributed logistic regression model was used to investigate associations between ten herd characteristics and high *Str. uberis* IMI PM.

Results

The national-level PM of *Str. uberis* IMI increased over time, from 16% (5 662 / 36 392) in 2013 to 17% (10 426 / 62 134) in 2018 and 19% (11 834 / 62 043) in 2023. From the sample of 1 839 herds, 1 501 (82%) were classified as 'normal/low' and 338 (18%) as 'high' *Str. uberis* IMI PM herds. The odds of high *Str. uberis* PM in a herd were associated with herd size, milking system, SCC, and year (Table 1). According to these results, a herd was less likely to be a high *Str. uberis* IMI PM herd in 2013 compared to 2023. However, no significant difference was found between 2023 and 2018.

Discussion

The preliminary results show a positive association between herd size and high *Str. uberis* PM. A greater susceptibility in larger herds suggests that a growing herd size could lead to changes in management practices which results in an increased likelihood of *Str. uberis* transmission.

Milking system has been recognized as a potential route for pathogen transmission (Wente et al., 2019). Previous results on the association between *Str. uberis* and milking system have been inconsistent (Riekerink et al., 2008; Smistad et al., 2023; Taponen et al., 2017). In automatic milking system (AMS), cows are milked in a random order instead of a structured one, which may predispose to greater pathogen transmission. This could potentially result in high *Str. uberis* PM, especially in cases of contagious-type strains. However, this study did not find such an association. Conversely, parlor herds were less likely to have high *Str. uberis* PM than herds with pipeline milking. As stall type was included in the milking system factor, this also suggests that herds in tie-stall housing have a greater susceptibility to have high *Str. uberis* PM compared to herds in free stall housing.

The observed positive association with annual herd-average SCC of individual cows' SCC measurements seems logical, since a high PM of a certain mastitis pathogen suggests a potential failure in prevention or management, which allows the specific pathogen to thrive. Consequently, a greater mastitis burden in a herd is likely to cause an increase in SCC. However, this relationship is not so straightforward, since an increase in herd-level SCC can also result from a variety of other factors and pathogens. Bulk tank SCC has been linked to several management factors (Chearbhaill et al., 2025). Thus, the observed association could be influenced by a management factor not represented in the data.

Conclusion

These preliminary results suggest that increasing herd size may create environmental hotspots for *Str. uberis* due to an association found between increasing average herd size and high PM. While no significant association was observed between *Str. uberis* PM

and automated milking systems compared with pipeline milking, parlor was associated with decreased susceptibility for high *Str. uberis* PM, indicating also higher susceptibility in tie-stall housing.

References

Chearbhaill, A. U., Boloña, P. S., Ryan, E. G., McAloon, C. I., McAloon, C. G., & Upton, J. (2025). Associations between on-farm factors and bulk tank SCC on Irish dairy farms. *Irish Veterinary Journal*, *78*(1).

Klaas, I. C., & Zadoks, R. N. (2018). An update on environmental mastitis: Challenging perceptions. *Transboundary and Emerging Diseases*, *65*, 166–185. https://doi.org/10.1111/tbed.12704

Riekerink, R. G. M. O., Barkema, H. W., Kelton, D. F., & Scholl, D. T. (2008). Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science*, *91*(4), 1366–1377. https://doi.org/10.3168/jds.2007-0757

Smistad, M., Bakka, H. C., Sølverød, L., Jørgensen, H. J., & Wolff, C. (2023). Prevalence of udder pathogens in milk samples from Norwegian dairy cows recorded in a national database in 2019 and 2020. *Acta Veterinaria Scandinavica*, *65*(1). https://doi.org/10.1186/s13028-023-00681-2

Srithanasuwan, A., Zou, Y., Suriyasathaporn, W., & Schukken, Y. H. (2025). Genetic diversity and molecular epidemiology of Streptococcus uberis in high-prevalence mastitis herds. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2025-26378

Taponen, S., Liski, E., Heikkilä, A. M., & Pyörälä, S. (2017). Factors associated with intramammary infection in dairy cows caused by coagulase-negative staphylococci, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Corynebacterium bovis, or Escherichia coli. *Journal of Dairy Science*, *100*(1), 493–503.

Wente, N., Klocke, D., Paduch, J. H., Zhang, Y., Seeth, M. tho, Zoche-Golob, V., Reinecke, F., Mohr, E., & Krömker, V. (2019). Associations between Streptococcus uberis strains from the animal environment and clinical bovine mastitis cases. *Journal of Dairy Science*, *102*(10), 9360–9369.

Table 1

Results from Logistic Regression Model for the Odds That the Herd's Annual Streptococcus uberis Intramammary Infection Proportional Morbidity is ≥ 19 % (n=1839).

Variable	group	β_i	OR	95 % CI	SE	P-value
Intercept		-1.41	0.24	0.19 - 0.32	0.13	<0.0001
SCC (x 10 000 cells/mL)[1,2]		0.08	1.09	1.07-1.11	0.01	<0.0001
Herd size (x 10 cows)[1]		0.06	1.07	1.04 - 1.09	0.01	<0.0001
Milking system						0.041
	AMS[3]	-0.28	0.76	0.54 - 1.06	0.17	0.10
	Parlor	-0.49	0.61	0.41 - 0.9	0.20	0.01
	Pipeline	Reference				
Year						<0.0001
	2013	-0.85	0.43	0.25 - 0.7	0.26	0.001
	2018	0.22	1.24	0.95 - 1.63	0.14	0.12
	2023	Reference				

[1]Tied to mean

[2]Annual herd-average SCC of individual cows' SCC measurements

[3]Automatic milking system

Antimicrobial resistance in bovine mastitis isolates of *Pasteurella multocida* and *Mannheimia haemolytica*

Wolfram Petzl[1], Lea Pirner [1,2] and Ulrike Sorge[2*]

[1] Clinic for Ruminants with Ambulatory and Herd Health Services, Centre for Clinical Veterinary Medicine, Ludwig Maximilians University Munich, Germany

[2] Department of Udder Health and Milk Quality, Bavarian Animal Health Services, Poing, Germany;

***Corresponding author's email: wpetzl@lmu.de**

Introduction

Pasteurella (*P.*) *multocida* and *Mannheimia* (*M.*) *haemolytica* are not primarily known as mastitis pathogens. However, they are usually associated with bovine respiratory disease (BRD). *P. multocida* are commonly found as bovine nasopharyngeal commensals and opportunistic pathogens. In contrast, *M. haemolytica* is considered the most significant pathogen of the BRD complex due to its virulence factors causing high morbidity (Noyes et al., 2015). Cases of mastitis caused by these two pathogens are rare. *P. multocida* mastitis has been primarily reported in case studies (Barnum, 1954), while *M. haemolytica* is more commonly associated with mastitis in sheep. Although the source of infection remains unknown, the upper respiratory system of calves and lambs has been suggested as a potential reservoir for both pathogens. Transmission occurs during suckling, and reports have indicated an increase in intramammary infections with *Pasteurella* or *Mannheimia spp*. in herds with nurse-cows. Unfortunately, due to the rarity of these infections, data on the antimicrobial resistance (AMR) profiles of *P. multocida* and *M. haemolytica* isolated from bovine mastitis are scarce. Conclusive data on AMR profiles are primarily available from isolates from BRD. This retrospective study aimed to assess the *in-vitro* AMR of *P. multocida* and *M. haemolytica* isolated from bovine mastitis in Bavaria, Germany, from 2015 to 2023.

Material & methods

All quarter milk samples submitted to the laboratory of the Bavarian Animal Health Services e. V. (TGD) between 2015 and 2023 were included in the analysis. These samples were collected by technicians during herd screenings or by veterinarians and farmers. The samples were visually inspected for signs of clinical mastitis and scored by California Mastitis Test (CMT). The milk was aseptically collected in 9 ml sample tubes containing boric acid and shipped to the laboratory. The samples were processed in accordance with the German Veterinary Association's (DVG) Guidelines.

Quarter milk samples were inoculated onto one quarter of an Aesculin-blood-agar plate. The plates were incubated at 36 ± 1°C for 18-24 hours and monitored for cultural growth. Colonies formed were evaluated by colony forming units and morphology. Gram-negative rods with colony morphology fitting *P. multocida* or *M. haemolytica* were differentiated using classic biochemical differentiation methods and MALDI-TOF-MS to determine the bacterial species. The pathogens' AMR was assessed by breakpoint analysis using a broth microdilution (breakpoint method, Micronaut-S-System). Multidrug resistance (MDR) was defined as isolates resistant to more than one antimicrobial. Statistical analysis was conducted in SAS 9.4. To summarize breakpoint observations, PROC FREQ procedures were used by year for each pathogen and mastitis status. Differences in MIC distributions and the odds ratio of each pathogen-antimicrobial-combination were compared by year (Chi-square). Only unadjusted p-values from the PROC FREQ procedures were reported. Cochran Armitage was used for trend analysis across all years (PROC FREQ).

Results & Discussion

Between 2015 and 2023, a total of 3,503,410 quarter milk samples were analyzed from 757,562 cows and 17,929 herds. During this 9-year period, 319 samples from 223 herds were analyzed using breakpoint analysis for either *P. multocida* or *M. haemolytica*.

The majority of isolates (94.4%, n=294) were single-isolates per cow, herd, and sampling. *P. multocida* was isolated more frequently (n=280), with a slight increase in the number of positive samples over the 9 years (p=0.05). In contrast, *M. haemolytica* (n=39) was rarely isolated each year, and no change in the number of isolates was observed over the study period. *P. multocida* was isolated almost as frequently from clinical (48.6%), as from subclinical mastitis cases (51.1%), while samples with *M. haemolytica* originated predominantly from clinical mastitis cases (82%).

Various studies on isolates from quarter milk samples and BRD samples have reported an increasing incidence of MDR, particularly for *P. multocida* (Kehrenberg et al., 2001). Most of the isolates proved to be susceptible to penicillin and studies show that the pathogens were eliminated from the udder after treatment (Maplesden & Carter, 1955). However there a no studies on potential self-cure rates of infected quarters like with other Gram-negative mastitis pathogens. *P. multocida* exhibited significant resistance to erythromycin and pirlimycin in our study. Consequently, a substantial portion of isolates also demonstrated multidrug resistance (MDR) against these two antimicrobials. Similarly, >40% of *M. haemolytica* isolates exhibited MDR against three antimicrobials: oxacillin, erythromycin, and pirlimycin.

Conclusion

Although the isolates in this study demonstrated a high risk of resistance to several tested antimicrobials, there was no overall increase in the *in-vitro* antimicrobial resistance of *P. multocida* and *M. haemolytica* throughout the study period. With regards to antimicrobial treatment, penicillin appears to be a potentially effective antimicrobial however there is a lack of knowledge concerning self-cure rates.

References

Barnum, D. A. (1954). A Herd Outbreak of Mastitis Caused by Pasteurella Multocida. *Can J Comp Med Vet Sci*, *18*(4), 113-119. https://www.ncbi.nlm.nih.gov/pubmed/17648709

Kehrenberg, C., Schulze-Tanzil, G., Martel, J. L., Chaslus-Dancla, E., & Schwarz, S. (2001). Antimicrobial resistance in pasteurella and mannheimia: epidemiology and genetic basis. *Vet Res*, *32*(3-4), 323-339. https://doi.org/10.1051/vetres:2001128

Maplesden, D. C., & Carter, G. R. (1955). Bovine Mastitis Caused By Pasteurella Haemolytica: A Case Report. *Can J Comp Med Vet Sci*, *19*(9), 295-296. https://www.ncbi.nlm.nih.gov/pubmed/17648849

Noyes, N. R., Benedict, K. M., Gow, S. P., Booker, C. W., Hannon, S. J., McAllister, T. A., & Morley, P. S. (2015). Mannheimia haemolytica in feedlot cattle: prevalence of recovery and associations with antimicrobial use, resistance, and health outcomes. *J Vet Intern Med*, *29*(2), 705-713. https://doi.org/10.1111/jvim.12547

Beyond the Questionnaire: Advancing the Understanding of Farmers' Decision-Making in Mastitis Management through Integrated Methods

Nina Lind[1*], Gabriela Olmos Antillón[2], Dorota Anglart[3], Nils Fall[2] and Lisa Ekman[2,4]
[1]Department of Economics, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[2]Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[3] DeLaval International AB, Tumba, Sweden
[4]Växa Sverige, Stockholm, Sweden
***Corresponding author's email: nina.lind@slu.se**

Thematic area: Udder Health

Introduction

Farmers' decisions play a crucial role in animal health management, yet our understanding of these decisions is often constrained by the methods we use to study them. Research on mastitis management has largely relied on surveys and self-reported data to capture farmers' decision-making, attitudes, intentions, and self-perceived control (e.g., (Biesheuvel et al., 2021; Jansen, 2010). These approaches have generated valuable population-level insights, but they also risk presenting a partial picture. When we rely solely on what farmers say or intend, we may overlook how actual herd health conditions and contextual realities shape those responses.

Understanding farmers' decisions thus requires not only listening to their perspectives but also linking these perspectives to observable outcomes and situational meanings. For instance, while questionnaires can describe behavioural patterns and psychological constructs, they cannot reveal how objective animal health data (e.g., somatic cell count, SCC) might influence or contradict those perceptions, nor how technological systems such as automatic milking systems (AMS) mediate daily practices. Similarly,

statistical associations may miss the experiential dimensions of decision-making that interviews can uncover.

This extended abstract problematises the limitations of relying on single-method research to understand complex on-farm decision processes. Drawing on recent work combining survey data, herd-level register data, and in-depth farmer interviews (Ekman, Anglart, et al., 2025; Ekman, Fall, et al., 2025), we argue that integrating these approaches can reveal layers of understanding that are otherwise obscured. By using mastitis as a case example, we show how methodological triangulation allows us to move from describing behaviour to understanding it, linking what farmers report, what their herds reveal, and what their stories explain.

Material & methods

Questionnaires as a Population Lens

One component consists of a large-scale cross-sectional survey distributed to Swedish dairy farmers using automatic milking systems (AMS) (Ekman, Fall, et al., 2025). Questionnaires provide a population-level overview, capturing the diversity of attitudes, motivations, and intentions within the sector. They quantify relationships between constructs such as risk perception, perceived control, and behavioural intentions. In this example, farmers responded to mastitis-related scenarios and analysed their health seeking behavior using Leventhal's Self-Regulation Model of Illness as a theoretical lens (Leventhal, 1984).

However, using questionnaires only means relying on self-reported data, shaped by memory, ideals, and interpretation. Responses may not always reflect on-farm realities. Without external validation, it remains unclear how closely reported perceptions align with actual herd health, highlighting the value of complementing subjective data with objective measures.

Registers as an Objective Anchor

An additional component adds national register data, in this specific case, average somatic cell count (SCC) was used as a proxy for udder health on the farm. These data provide an objective factual anchor that enables comparison between farmers' perceptions and measurable herd health outcomes. Linking register and survey data allows exploration of whether herd performance influences how farmers perceive udder health.

This integration exposes perceptual gaps, for example, cases where farmers perceive good udder health despite high SCC levels, revealing where advisory attention may be needed. While register data quantify outcomes, they cannot explain why differences occur. Their true value lies in combination: aligning self-perceived and objective health measures to identify where perception, knowledge, and behaviour diverge.

Interviews as a Meaning-Making Tool

Adding a qualitative component is a crucial pillar to a holistic view. In this example this involves semi-structured interviews with farmers representing diverse herd sizes, production systems, and AMS usage (Ekman, Anglart, et al., 2025). Interviews reveal the meanings farmers attach to technology, data, and animal care, uncovering how experience, values, and constraints shape everyday decision-making.

Beyond serving as an independent source of insight, interviews also help interpret quantitative findings. They clarify why certain patterns appear in surveys or registers, such as why some farmers trust behavioural cues more than AMS alerts, and how "control" or "good management" are understood in context. Through dialogue, farmers co-interpret results, turning data into shared understanding and linking measurement with meaning.

Results & Discussion

Problematizing the Single-Method Approach

When taken in isolation, each methodological approach tells only part of the story. Surveys describe patterns but risk abstraction from real-life complexity. Registers quantify herd outcomes but cannot explain behavioural reasoning. Interviews reveal meanings but lack population-level generalisability. Relying on any single method limits our capacity to interpret farmers' decision-making as a situated and multifaceted process. For example, survey data alone might suggest that perceived control predicts treatment intentions. Yet, without register data, we cannot know whether this sense of control corresponds to actual herd health outcomes, or whether it represents overconfidence or underestimation. Similarly, interview narratives can suggest why farmers act in certain ways, but without population data, we cannot gauge whether these narratives are typical or exceptional.

Gaining Insight through Integration

The strength of a multi-method design lies in its capacity to interconnect these partial perspectives. When self-reported perceptions are compared with herd registers, discrepancies become visible: some farmers perceive their herd's udder health as good despite high SCC levels, while others underestimate their performance. Understanding these mismatches can help tailor communication strategies that align advisory messages with farmers' lived perceptions.

Adding interviews further enriches interpretation. For instance, farmers' reliance on behavioural cues (e.g., cows being late for milking) rather than AMS health alerts only, reveals a logic grounded in familiarity and embodied observation rather than technological data alone. This helps explain why some farmers' perceptions deviate from recorded herd health measures, not as irrationality, but as a function of trust, experience, and context.

Thus, integrating multiple data sources moves research from describing variance to understanding coherence: how perception, action, and outcome interrelate. It also allows researchers to move beyond surface-level correlations and to interpret farmers' responses as expressions of meaning-making within specific environments.

Towards Methodological Reflexivity in Animal Health Research

The challenge of understanding farmers' decisions is not only empirical but also epistemological: what counts as knowledge about behaviour? By extending methodological designs, researchers acknowledge that different data types reveal different truths. Questionnaires give breadth, registers give objectivity, and interviews

give depth. Their combination allows triangulation that both strengthens validity and deepens interpretation.

In the field of animal health, such reflexivity is essential. Decisions about mastitis management are influenced by economic, ethical, and emotional considerations by the farmer that cannot be reduced to numbers alone. Integrating quantitative and qualitative evidence encourages advisory systems and researchers alike to treat farmers as knowledgeable partners rather than data points, co-creators of understanding rather than passive subjects.

Conclusion

When we rely only on one method we risk mistaking intentions for actions. By combining surveys, register data, and interviews, we can move beyond description to explanation, seeing how farmers' perceptions, herd realities, and lived experiences intersect which can transform our understanding of what it means to make decisions about animal health.

For mastitis management, methodological integration offers both a scientific and practical gain: it allows researchers, veterinarians, and farmers to see the same problem from multiple vantage points, assembling the puzzle rather than examining a single piece. Future work should continue to build such bridges, linking behavioural theory with real-world data and participatory dialogue, to advance both animal welfare and the human understanding that sustains it.

References

Biesheuvel, M. M., Santman-Berends, I. M. G. A., Barkema, H. W., Ritter, C., Berezowski, J., Guelbenzu, M., & Kaler, J. (2021). Understanding Farmers' Behavior and Their Decision-Making Process in the Context of Cattle Diseases: A Review of Theories and Approaches. Frontiers in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.687699

Ekman, L., Anglart, D., Gillsjö, I., Lind, N., Fall, N., & Antillón, G. O. (2025). What information counts when detecting mastitis in automatic milking systems? A mixed methods approach from a Swedish perspective. Journal of Dairy Science, 108(9), 9861–9875. https://doi.org/10.3168/jds.2025-26455

Ekman, L., Fall, N., Emanuelson, U., & Lind, N. (2025). Farmer attitudes and motivation affect their health-seeking behavior in relation to mastitis in dairy cows—A survey on Swedish dairy farms with automatic milking systems. Journal of Dairy Science. https://doi.org/10.3168/jds.2025-27187

Jansen, J. (2010). Mastitis and farmer mindset. Towards Effective Communication Strategies to Improve Udder Health Management on Dutch Dairy Farms.

Leventhal, E. A. (1984). Aging and the perception of illness. Research on Aging, 6(1), 119–135.

RealPCR* MilQ-ID* DNA System

RealPCR MilQ-ID DNA System: The new solution for mastitis testing

Martina Kahila [1], Loic Commun[1], Katharina Engelke[1], Christoph Egli[1], Kathy Velek[2]

[1] IDEXX Europe, Hoofddorp, The Netherlands
[2] IDEXX Laboratories Inc, Westbrook, Maine, USA
***Corresponding author's email: martina-kahila@idexx.com**

Introduction

Mastitis is the most frequent infectious disease with the most important economic consequences in dairy cattle (1). The identification of mastitis-causing pathogens is key to adapting treatments and reducing the number of cases. These causative pathogens were historically identified by culture (2), but today, the RealPCR* MilQ-ID DNA System, for use with milk samples, allows for identification of 4–16 targets in a single test run in 3 hours.

Material & methods

Studies were conducted to determine the performance of the RealPCR* MilQ-ID DNA System in synthetic or clinical samples. Analytical sensitivity and PCR efficiency were determined through testing dilutions of synthetic DNA representing the RealPCR* MilQ-ID DNA System targets. Log dilutions in the range of 10,000,000 copies to 1 copy per 25 µL reaction were prepared, and multiple replicates of each dilution were tested with the DNA mixes, using standard test reagents and protocol. The analytical sensitivity limit of detection (LD_{PCR}) is the smallest number of target nucleic acids per reaction, detectable in at least 60% of the test results. PCR efficiency is calculated as $(10^{(-1/slope)}-1) \times 100$ over a 7-log range plotted from one session of testing.

Results & Discussion

Table 1 Performance data of the four MilQ-ID mixes

Target	LD_{PCR} (copies/reaction)	PCR % Efficiency
MilQ-ID DNA Mix 1		

M. bovis	1 copy	104.5%
S. aureus	1 copy	108.9%
S. uberis	1 copy	103.0%
S. agalactiae	1 copy	105.6%
MilQ-ID DNA Mix 2		
S. dysgalactiae	1 copy	100.5%
ß-lactamase	10 copies	99.4%
E. coli	10 copies	98.6%
Staphylococcus spp.	10 copies	106.3%
MilQ-ID DNA Mix 3		
T. pyogenes	10 copies	98.0%
Enterococcus spp.	10 copies	98.7%
Prototheca spp.	1 copy	97.8%
Klebsiella spp.	1 copy	99.7%
MilQ-ID DNA Mix 4		
Mycoplasma spp.	10 copies	99.8%
Pseudomonas aeruginosa	1 copy	101.6%
Yeast	10 copies	90.2%
Corynebacterium bovis	1 copy	106.2%

Each of the MilQ-ID DNA targets was detectable at 1 or 10 copies per reaction, which demonstrates excellent sensitivity for the MilQ-ID DNA System.

Conclusion

The RealPCR*R MilQ-ID DNA System offers a new, quick, and accurate tool for the management of mastitis at farm level. The RealPCR* MilQ-ID DNA System is changing the game in mastitis testing:

- Less than 3 hours turnaround time in the laboratory with excellent accuracy
- Modular platform for flexible combination of targets on the same PCR run
- Cloud-based RealPCR Connect* software for results interpretation and reporting
- Internal positive control detects extraction or PCR failure

References

1. Halasa T, Huijps K, Østerås O, Hogeveen H. (2007). Economic effects of bovine mastitis and mastitis management. *Vet Q.* 29(1):18-31.
2. Poutrel B. Is bacteriological diagnosis of mastitis by PCR reliable? (2019). *Bulletin G.T.V*. 93:73-78.

Dysbiosis of the udder microbiota as a risk factor for mastitis in cows

Meruyert Alyonova[1,2], Raikhan Mustafina[1,2], Leila Sultanayeva[1,2], Galia Zamaratskaia[3]*, Yuriy Balji[1, 2]

[1]Faculty of Veterinary and Animal Husbandry Technology, Kazakh Agrotechnical Research University named after S. Seifullin, Astana, Republic of Kazakhstan
[2]LLP "NFT-KATU", Astana, Republic of Kazakhstan
[3]Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

***Corresponding author's email: galia.zamaratskaia@slu.se**

Introduction

Bovine mastitis remains one of the most prevalent and economically important diseases in dairy farming, leading to reduced milk yield, lower milk quality, and higher treatment costs. This problem is particularly pronounced in parts of Central Asia, including Kazakhstan, where both clinical and subclinical forms occur at high levels in commercial herds. On dairy farms in East Kazakhstan, clinical mastitis affected up to 35% of cows in some years, with subclinical mastitis prevalence similarly high, indicating that the disease remains widespread despite ongoing prevention efforts (Mukhamadieva et al., 2023). The high incidence of udder inflammation in this region reflects broader challenges in feeding, housing, and herd management, making mastitis a persistent health and economic concern for local dairy farmers.

Recent research has emphasized the important role of the mammary gland microbiota in maintaining udder health and in the development of inflammatory disorders. Advances in next-generation sequencing (NGS) techniques have enabled detailed profiling of microbial communities, including bacteria that cannot be cultured traditionally, providing new insights into the microbial dynamics underlying mastitis (Derakhshani et al., 2018). The present study aimed to investigate changes in the composition and structure of the mammary microbiota in dairy cows across healthy, subclinical, and clinical mastitis conditions.

Material & methods

The study was conducted on one commercial dairy farm located in the Akmola region (Central Kazakhstan). A total of 30 dairy cows were included and divided into three experimental groups: clinically healthy cows, cows with subclinical mastitis, and cows with clinical mastitis (10 cows per group). Milk sampling was performed at the cow level, with one individual milk sample collected from each cow, resulting in a total of 30 milk samples. Clinical mastitis was diagnosed based on veterinary clinical examination, the California Mastitis Test (CMT; DeLaval, Sweden), and direct determination of somatic cell count (SCC) using the fluoro-opto-electronic method in accordance with ISO 13366-2 | IDF 148-2:2006. Subclinical mastitis was defined solely based on SCC, with a threshold value exceeding 400,000 cells/mL. Before milk sampling, the udder was washed with water, treated with a pre-milking disinfectant (Kenopur), and dried using disposable towels. No additional antiseptic treatment of teat surfaces was applied prior to sampling. The first streams of milk were discarded, after which 100 mL of milk was aseptically collected into sterile disposable containers while wearing gloves. Milk samples were transported to the laboratory within 2 hours after collection and stored at 4-5 °C until analysis. Samples were not frozen.

Bacterial identification was performed by direct nucleotide sequencing of the 16S rRNA gene, followed by comparison of nucleotide identity with sequences deposited in the international GenBank database. Phylogenetic trees were constructed using nucleotide sequences of reference strains.

Ethical statement

All experimental procedures involving animals were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals (2012) and complied with the ethical standards of Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes. The study protocol was approved by the local ethics committee (Protocol No. 2, dated 01 November 2023). Milk sampling was performed with the knowledge and consent of the farm management.

Results & Discussion

Analysis of the taxonomic structure at the phylum level revealed pronounced differences among clinically healthy cows, cows with subclinical mastitis, and cows with clinical mastitis (Table 1). In all groups, the dominant phylum was *Firmicutes*, consistent with previous reports (Derakhshani et al., 2018). The relative abundance of *Firmicutes* was highest in cows with subclinical mastitis (73.4%), whereas cows with clinical mastitis showed a lower relative abundance (57.4%). This lower representation of *Firmicutes* in clinical mastitis may reflect a disruption of microbial community stability and a shift toward other taxa, as reduced *Firmicutes* abundance has been associated with dysbiosis in mastitic milk (Falentin et al., 2016).

Table 1. Microbiome composition by mastitis status based on 16S rRNA analysis.

Taxon (Phylum)	Healthy	Subclinical mastitis	Clinical mastitis
Firmicutes, %	68.8	73.4	57.4
Bacteroidota, %	13.1	16.6	5.4
Proteobacteria, %	7.0	1.4	11.2
Actinobacteriota, %	4.0	2.4	11.4
Bdellovibrionota, %	1.7	0.3	13.4
Other phyla, %	5.3	6.0	1.2
Total diversity (number of phyla)	14	14	11

The phylum *Bacteroidota* ranked second in relative abundance in healthy cows (13.1%) and in cows with subclinical mastitis (16.6%), whereas cows with clinical mastitis exhibited a lower relative abundance (5.4%). Members of *Bacteroidota* are frequently associated with commensal microbial communities, and lower relative abundances of this phylum have been reported in association with dysbiosis and inflammatory conditions of the mammary gland (Zhu et al., 2024).

Considerable differences were also observed in the relative abundance of *Proteobacteria*. In healthy animals, *Proteobacteria* accounted for 7.0% of sequences, were less abundant in cows with subclinical mastitis (1.4%), and were most abundant in cows with clinical mastitis (11.2%). Many members of *Proteobacteria* are opportunistic or pathogenic microorganisms, and a higher relative abundance of this phylum has been associated with inflammation and microbial instability in disease.

A similar pattern was observed for *Actinobacteriota*. Higher relative abundances of *Actinobacteriota* have been reported in association with dysbiotic conditions and may reflect either the opportunistic proliferation of specific genera or changes in local microenvironmental conditions associated with inflammation (Derakhshani et al., 2018).

Of particular interest was the phylum *Bdellovibrionota*, which reached a relative abundance of 13.4% in clinical mastitis, compared with 1.7% in healthy cows and 0.3% in subclinical mastitis. Members of *Bdellovibrionota* are often described as bacterial predators in environmental ecosystems, and their presence at elevated relative abundance may reflect restructuring of the microbial community under conditions of dysbiosis. To the best of our knowledge, there are no reports documenting *Bdellovibrionota* in the bovine milk microbiome specifically. However, elevated levels of minor or predatory taxa have been noted in other dysbiotic microbiomes (Lam and Ye, 2022).

Overall taxonomic diversity also differed among health states: 14 bacterial phyla were identified in both healthy cows and cows with subclinical mastitis, whereas only 11 phyla were detected in cows with clinical mastitis. Reduced diversity is a characteristic

feature of inflammatory diseases and reflects a loss of resilience and stability in the microbiota (Derakhshani et al., 2018).

Conclusion

The present study demonstrated that bovine mastitis was characterized by distinct differences in the composition and diversity of the mammary gland microbiota at the phylum level. *Firmicutes*, the dominant phylum in healthy and subclinical cows, showed a marked reduction in clinical mastitis. *Bacteroidota*, typically associated with commensal communities, was also reduced in clinical mastitis. In contrast, opportunistic or potentially pathogenic phyla, including *Proteobacteria* and *Actinobacteriota*, were more abundant in clinical mastitis. *Bdellovibrionota*, a phylum of predatory bacteria rarely reported in milk, reached elevated levels in clinical mastitis. Overall, taxonomic diversity declined in clinical mastitis compared with healthy and subclinical states.

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References

Derakhshani, H., Fehr, K. B., Sepehri, S., Francoz, D., De Buck, J., Barkema, H. W., Plaizier, J. C., & Khafipour, E. (2018). Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. Journal of dairy science, 101(12), 10605–10625.

Falentin, H., Rault, L., Nicolas, A., Bouchard, D. S., Lassalas, J., Lamberton, P., Aubry, J.-M., Marnet, P.-G., Le Loir, Y., & Even, S. (2016). Bovine teat microbiome analysis revealed reduced alpha diversity and significant changes in taxonomic profiles in quarters with a history of mastitis. Frontiers in Microbiology, 7, Article 480.

Lam, T. J., & Ye, Y. (2022). Meta-analysis of microbiome association networks reveal patterns of dysbiosis in diseased microbiomes. *Scientific Reports, 12*, 17482.

Mukhamadieva, N., Zainettinova, D., Julanov, M., Stefanik, V., Nurzhumanova, Z., Julanova, N., Alimbekova, M., & Akzhigitov, N. (2023). Study of mastitis incidence in cows of dairy farms in East Kazakhstan: Impacts of nutrition, endometritis and mycotoxin contamination. American Journal of Animal and Veterinary Sciences, 18(4), 292–303.

Taponen, S., McGuinness, D., Hiitiö, H., Simojoki, H., Zadoks, R., & Pyörälä, S. (2019). Bovine milk microbiome: a more complex issue than expected. Veterinary research, 50(1), 44.

Zhu, C., Zhao, Y., Yang, F., Zhang, Q., Zhao, X., Yang, Z., Dao, X., & Laghi, L. (2024). Microbiome and metabolome analyses of milk and feces from dairy cows with healthy, subclinical, and clinical mastitis. Frontiers in Microbiology, 15, 1374911.

Mastitis Management: Knowledge, Attitudes, and Practices in Tropical Dairy Farms

Giovanny Torres-Lindarte[1*], Julián Reyes-Vélez[1], Mónica Jiménez-Serna[1], Arley Caraballo-Guzmán[1]

[1]Colombian Institute of Tropical Medicine-CES University, Sabaneta, Antioquia, Colombia

***Corresponding author's email: gtorres@ces.edu.co**

Introduction

Colombia ranks fourth as a milk producer in Latin America, with an annual production of approximately 7 billion liters, with Antioquia contributing about 20% as the leading region. About 80% of this production comes from smallholder farmers (< 50 cows), characterized by low levels of milking mechanization and limited technical capacity (Taramuel-Taramuel et al., 2025).

Antimicrobial use (AMU) in food-producing animals represents almost 70% of global consumption, with dairy systems contributing substantially, particularly through the prevention and treatment of intramammary infections (Mulchandani et al., 2023). Inappropriate AMU, driven by variable decision-making, limited diagnostics, weak surveillance, and insufficient stewardship, contributes to antimicrobial resistance (AMR), a growing global concern, especially in South America (Kallu et al., 2024; Mulchandani et al., 2023).

The Knowledge, Attitude, and Practice (KAP) model offers a standardized approach to identify gaps affecting antimicrobial stewardship (Zarei et al., 2024). However, determinants of KAP in tropical dairy systems remain poorly understood. This study aimed to characterize KAP related to bovine mastitis, bacteriological culture, and AMU in tropical dairy farms and to assess their association with sociodemographic factors.

Material & methods

A descriptive observational study was conducted between August and December 2023 among 366 decision-makers (owners, administrators and/or managers) of dairy farms located in five municipalities of Antioquia. A digital KAP survey was administered.

The questionnaire was developed through a structured participatory process involving subject-matter experts and an extensive review of the scientific literature. The 71

defined questions were organized into sociodemographic variables and KAP components related to bovine mastitis diagnosis, information management, and AMU.
The study received ethical approval from the Colombian Institute of Tropical Medicine (Minutes No. 77 of the Ethics Committee), and informed consent was obtained from all participants. For statistical analysis, single-response KAP questions were scored using a standardized system: 0 corresponding to poor, 0.5 to moderate, and 1 to good, generating total scores and categorical classifications (good, moderate and poor) based on percentile distribution (Ratanapob et al., 2024). Descriptive analyses were performed, Spearman correlation was used to assess relationships among KAP scores, and ordinal logistic regression models were applied to identify associations with sociodemographic variables.

Results & Discussion

More than half of respondents (56.6%) reported never using microbiological culture for mastitis diagnosis. According to other studies, the main barriers to the use of microbiological culture were cost, delays in obtaining results, and limited awareness of its value for guiding therapeutic decisions (de Jong et al., 2024; Farrell et al., 2023).
Antimicrobials were widely accessible (98.6%); however, only 34.3% of participants routinely consulted a veterinarian before administering them, and fewer than half (41.3%) kept records of antimicrobial use. Producers commonly seek veterinary advice only after initial treatments fail, as reported by the majority of respondents (58.1%). This behavior has also been documented in earlier studies, where delaying veterinary consultation is perceived as a cost-saving strategy (Farrell et al., 2023; Kallu et al., 2024). In addition, blanket dry cow therapy was a common practice, reported by 81.2% of producers. The growing global pressure to reduce AMU in livestock production has led several countries, particularly in Europe, to implement measures that restrict their use for preventive purposes and to strengthen antimicrobial stewardship programs (Borelli et al., 2023; Llanos-Soto et al., 2021).
Analysis of KAP scores showed positive correlations between knowledge, attitudes, and practices among dairy farms, with the strongest association observed between knowledge and practices (correlation coefficient 0.7012), followed by knowledge and attitude (0.5130), and attitude and practices (0.4981), all statistically significant ($p < 0.05$). Higher KAP scores were consistently associated with larger herd sizes and greater producer experience, whereas lower educational levels and the use of manual milking systems were linked to poorer performance across all KAP dimensions (Table 1).

Table 1. Univariate ordinal regressions showing statistically significant associations with KAP scores

Variable	Knowledge		Attitudes		Practices	
	OR	*p* > z	OR	*p* > z	OR	*p* > z
Education level						
Primary	0.109	< 0.001	0.274	0.015	0.150	< 0.001
High School	0.195	0.001	0.257	0.008	0.203	0.001
Milking type						
Manual	0.123	< 0.001	0.231	< 0.001	0.078	0.001
Experience years						
10-15 years	2.492	0.005	2.359	0.011	2.088	0.027
15-20 years	3.363	< 0.001	1.962	0.036	3.398	< 0.001
Total cows						

51-100	2.655	< 0.001			3.074	< 0.001
>100	4.161	< 0.001	2.692	< 0.001	3.896	< 0.001

OR: odds ratio; *p*: *p*-value (< 0.005)

Although most participants have heard about AMR (88.0%), half of the respondents (50.5%) did not perceive AMR as a relevant threat to their farms, highlighting a critical gap between awareness and risk perception that may hinder effective antimicrobial stewardship. Limited awareness of AMR and the prioritization of individual benefits over collective responsibility contribute to antimicrobial misuse (Awosile & Smith, 2017; Borelli et al., 2023).

Conclusion

This study constitutes the first analysis in Colombia to apply a KAP approach to evaluate AMU in dairy production. The study highlights challenges in responsible AMU in dairy farming, linked to easy antimicrobial access, limited veterinary guidance, lack of standardized protocols, and knowledge gaps. Poorer practices were associated with lower education, manual milking, and small-scale farms.

These findings provide critical evidence to guide educational programs, strengthen regulations, and implement AMR control strategies in Colombia, a major milk producer. Aligning national policies with global AMR mitigation efforts is vital, as Colombia's limited controls enable inappropriate antimicrobial use compared to countries with stricter policies and robust surveillance.

References

Awosile, B. B., & Smith, B. A. (2017). Risk assessment modelling of fecal shedding caused by extended-spectrum cephalosporin-resistant Escherichia coli transmitted through waste milk fed to dairy pre-weaned calves. *Journal of Dairy Science*, *100*(12), 9667–9673. https://doi.org/10.3168/jds.2017-13196

Borelli, E., Ellis, K., Tomlinson, M., & Hotchkiss, E. (2023). Antimicrobial usage and resistance in scottish dairy herds: A survey of farmers' knowledge, behaviours and attitudes. *BMC Veterinary Research*, *19*(1), 72. https://doi.org/10.1186/s12917-023-03625-0

de Jong, E., McCubbin, K. D., Uyama, T., Brummelhuis, C., Bodaneze, J., Kelton, D. F., Dufour, S., Sanchez, J., Roy, J.-P., Heider, L. C., Rizzo, D., Léger, D., & Barkema, H. W. (2024). Adoption and decision factors regarding selective treatment of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science*, *107*(1), 476–488. https://doi.org/10.3168/jds.2023-23608

Farrell, S., Benson, T., McKernan, C., Regan, Á., Burrell, A. M. G., & Dean, M. (2023). Exploring veterinarians' behaviour relating to antibiotic use stewardship on Irish dairy farms using the COM-B model of behaviour change. *Research in Veterinary Science*, *156*, 45–53. https://doi.org/10.1016/j.rvsc.2023.01.019

Kallu, S. A., Kebede, N., Kassa, T., Wubaye, A. M., Kainga, H., Mekonnen, H., & Simuunza, M. C. (2024). Knowledge, Attitudes, Practices, and Risk Perception of Antimicrobial Use and Antimicrobial Resistance Among Dairy Farm Owners/Workers in Addis Ababa, Ethiopia. *Infection and Drug Resistance*, *17*, 1839–1861. https://doi.org/10.2147/IDR.S453570

Llanos-Soto, S. G., Vezeau, N., Wemette, M., Bulut, E., Greiner Safi, A., Moroni, P., Shapiro, M. A., & Ivanek, R. (2021). Survey of perceptions and attitudes of an international group of veterinarians regarding antibiotic use and resistance on dairy

cattle farms. *Preventive Veterinary Medicine*, *188*, 105253. https://doi.org/10.1016/j.prevetmed.2020.105253
Mulchandani, R., Wang, Y., Gilbert, M., & Boeckel, T. P. V. (2023). Global trends in antimicrobial use in food-producing animals: 2020 to 2030. *PLOS Global Public Health*, *3*(2), e0001305. https://doi.org/10.1371/journal.pgph.0001305
Ratanapob, N., Saengtienchai, A., & Rukkwamsuk, T. (2024). Knowledge, Attitude, and Practice of Thai Dairy Farmers on the Use of Antibiotics. *Veterinary Medicine International*, *2024*, 5553760. https://doi.org/10.1155/2024/5553760
Taramuel-Taramuel, J. P., Delgado-López, M. A., Aza-Fuelantala, O. E., & Barrios, D. (2025). Technological and socioeconomic characteristics of smallholder dairy farms in Indigenous Pastos communities of Colombia. *Tropical Animal Health and Production*, *57*(7), 363. https://doi.org/10.1007/s11250-025-04576-4
Zarei, F., Dehghani, A., Ratansiri, A., Ghaffari, M., Raina, S. K., Halimi, A., Rakhshanderou, S., Isamel, S. A., Amiri, P., Aminafshar, A., & Mosavi Jarrahi, A. (2024). ChecKAP: A Checklist for Reporting a Knowledge, Attitude, and Practice (KAP) Study. *Asian Pacific Journal of Cancer Prevention: APJCP*, *25*(7), 2573–2577. https://doi.org/10.31557/APJCP.2024.25.7.2573

Genetic diversity of *Streptococcus agalactiae* in Norwegian bovine dairy herds

Marit Smistad[1]*, Hannah Joan Jørgensen[2], Camilla Sekse[3], Liv Synnøve Sølverød[1], and Solveig Sølverød Mo[3]

[1]TINE Mastitis Laboratory, Molde, Norway

[2]Norwegian Institute of Public Health, Oslo, Norway

[3]Norwegian Veterinary Institute, Ås, Norway

***Corresponding author's email: marit.smistad@tine.no**

Introduction

Streptococcus agalactiae (Group B Streptococcus, GBS) is a contagious udder pathogen causing chronic mastitis in dairy cows, leading to reduced milk yield and significant economic losses (Keefe, 1997). Although nearly eliminated in dairy cattle in Nordic countries through control programs in the 1950s-90s, *S. agalactiae* has since reappeared and remains a problem in some Norwegian herds. The difficulty in eradicating *S. agalactiae* coincides with structural changes in Norwegian dairy production, including larger herd sizes, transition to free-stall housing, increased animal movements, and discontinuation of active surveillance. Traditional control measures may be less effective in modern herds, and environmental survival and other strain-level differences may contribute to persistence (Jørgensen et al. 2015, Mahmmod et al., 2015, Sørensen et al., 2019). Understanding the distribution and characteristics of *S. agalactiae* strains is essential for developing targeted control strategies. The aim of this study was to explore the genetic diversity of *S. agalactiae* in Norwegian bovine dairy herds and explore herd-level factors and strains associated with the persistence of *S. agalactiae*.

Material & methods

S. agalactiae-isolates were collected from bovine milk samples at the national mastitis laboratories between 2008-2023. Herd-level data and bacteriology results were available from the Norwegian Dairy Herd Recording System (NDHRS), which covers 97% of the herds.

A total of 271 isolates from 132 different farms were characterized using multi-locus sequence typing (MLST, PCR and Sanger sequencing) and/or by whole genome sequencing (WGS). MLST identified sequence types (STs) based on seven housekeeping genes, while WGS enabled deeper analysis of genetic diversity, in addition to MLST. Bioinformatic analyses included detection of MLST, AMR genes and phylogenetic analyses.

Herds were categorized by infection duration into short duration (1–2 years) or long duration (≥3 years). Descriptive statistics summarized the number of detections per farm, the number of years with herd infection and compared herd characteristics (size, milking system, yield) between *S. agalactiae*-positive and all Norwegian herds, and between herds infected with the most common STs.

Results & Discussion

Based on data from the NDHRS, 450 herds had at least one detection of *S. agalactiae* between 2008–2023. Each year, between 50 and 80 herds had *S. agalactiae* detected, representing less than 1% of Norwegian dairy farms annually. *S. agalactiae*-positive herds had a mean size of 43 cows, compared to 32 on the national average in 2023. Most herds (76%) had short duration of herd infection (1–2 years). Long duration of herd infection was associated with larger herd size, which in Norway is correlated with free-stall housing and automatic milking systems.

A total of 30 different STs were identified in the 132 farms, reflecting relatively high genetic diversity at the national level and multiple independent introductions into the Norwegian dairy population. Together, ST1, ST23, ST103, and ST196 accounted for 66% of herd infections, indicating that these four STs have successfully established in the Norwegian dairy system.

The median number of years with herd infection was slightly higher for ST1 (5 years) compared to other sequence types (3 years for ST23, ST103, ST196, and other STs), suggesting that the duration of herd infection depends on factors other than ST.

Furthermore, a substantial proportion of the detected STs demonstrated potential for significant impact on udder health, spread, and long-term persistence in dairy herds: 14 STs (47%) were identified in multiple herds; 21 STs (70%) were found in farms with at least 10 detections per farm; and 12 STs (40%) were present in herds with five or more years of herd infection. No major differences in herd size, milking system, or yield were found between herds infected with the most common STs.

Within individual herds, genetic diversity was low: 84% of herds had only one circulating ST, while a minority had two, likely due to new introductions. Core genome SNP analysis revealed clustering of isolates from the same herd and generally limited rate of development over years. Some clusters included highly similar isolates from different farms, indicating an epidemiological link (Figure 1). The phylogeny also revealed several distinct clusters within each ST, suggesting that higher resolution than MLST is required to infer between-farm transmission of *S. agalactiae*.

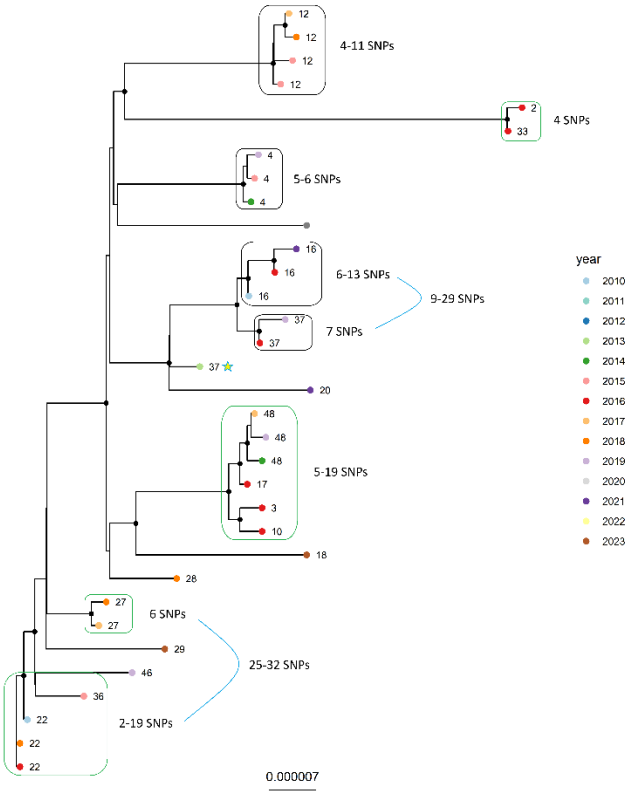

Figure 1: Core genome phylogeny of *Streptococcus agalactiae* sequence type 103 and ST2283 collected from Norwegian dairy herds from 2010-2023. Color of tippoints indicates year of collection, while the number indicates herd ID. Clusters marked with black boxes include isolates from a single farm, while clusters marked with green boxes include isolates from more than one farm. Number of SNP differences are indicated next to each cluster.

This pattern indicates that while new introductions do occur, they are relatively rare, and the main challenge for affected farms is not repeated introductions but rather the persistence and difficulties in eradication of established strains. This implies that general *S. agalactiae* control measures remain appropriate, and that persistent herd infections in Norway are more likely driven by herd-level factors than by inherent differences in strain virulence or persistence.

Conclusion

S. agalactiae remains a challenge in some Norwegian dairy herds, particularly in larger farms. The study found substantial genetic diversity among isolates at national level, with no evidence for the need for ST-specific control measures. Persistent infections are linked to herd size and milking system highlighting the need for tailored management strategies. Further research should focus on risk factors for introduction and persistence, and the role of herd management in controlling *S. agalactiae*.

References

Keefe, G. P. (1997). Streptococcus agalactiae mastitis: a review. The Canadian veterinary journal, 38(7), 429.

Jørgensen, H. J., Nordstoga, A. B., Sviland, S., Zadoks, R. N., Sølverød, L., Kvitle, B., & Mørk, T. (2016). Streptococcus agalactiae in the environment of bovine dairy herds–rewriting the textbooks? *Veterinary microbiology*, *184*, 64-72.

Mahmmod, Y. S., Klaas, I. C., Katholm, J., Lutton, M., & Zadoks, R. N. (2015). Molecular epidemiology and strain-specific characteristics of Streptococcus agalactiae at the herd and cow level. *Journal of dairy science*, *98*(10), 6913-6924.

Sørensen, U.B.S. et al. (2019). The distribution of clones of Streptococcus agalactiae among herdspersons and dairy cows demonstrates lack of host specificity for some lineages. Veterinary Microbiology, 235, 71–79.

MILKING TECHNOLOGY THEMATIC AREA

Photo: Mark Harris, SLU

Long Term Impact of Delayed Milk Ejection on Milk Yield

(Delayed milk ejection and milk yield)

Paola Bacigalupo Sanguesa[1,2], Pamela L. Ruegg [1], Roger Thomson [1], Bo Norby[1], and Ronald Erskine[1]

[1]Michigan State University, East Lansing, USA

[2] [1]Michigan State University Extension, East Lansing, USA

***Corresponding author's email: paolabs@msu.edu**

Introduction

Delayed milk ejection (DME) or bimodal milk letdown is the result of a disruption between the flow of alveolar and cisternal milk, caused by a failure to elicit the milk ejection reflex before milking (Bruckmaier & Blum, 1998). DME can have herd between 0 to more than 80% prevalences (Fernandes et al., 2023; Moore-Foster et al., 2019) and it is associated with negative impacts, including reduced milk yield in a single milking (Dahl & Wieland, 2025; Erskine et al., 2019). While the impact of DME on milk yield in a single milking has been studied, results have been contradictory (Bruckmaier & Blum, 1996; Kaskous & Bruckmaier, 2011; Tuor et al., 2023), and it remains unknown if the associated yield loss is transient or persists over time. This observational study aimed to evaluate the association between DME events and long-term milk yield in commercial dairy herds. We hypothesized that a higher frequency of DME events would be associated with a sustained reduction in accrued milk yield over a 10-day period.

Materials and Methods

The study was conducted on four commercial dairy farms milking three times per day. Data were collected over 10 consecutive days from cows between 21 and 365 days in milk (DIM). Individual milking session data, including yield and average milk flow rate between 30-60 seconds after unit attachment (FLOW30-60), were collected from on-farm software. A DME event was defined as a FLOW30-60 of <1.8 kg/min. For each cow, the percentage of DME events over the 10-day study period was calculated and categorized (DME%CAT). Total milk yield over 10 days (10YIELD) was the sum of all session yields and was the outcome variable. A multivariable linear regression analysis was performed to assess the association between 10YIELD and DME%CAT while

controlling for the fixed effects of farm, lactation, and stage of lactation curve (based on DIM), and their interactions with DME%CAT.

The ls-means for the extreme DME%CAT of 0-10% and 91-100% were 426.8 and 338.9 kg, respectively. Our results showed that cows with more milkings with DME were associated with greater reduction in accrued milk yield. Dairy farms should make efforts to prevent DME and avoid losses in milk yield.

Results & Discussion

The final dataset included 8,447 cows. The individual percentage of DME ranged from 0% for cows that did not have a single DME episode to 100%, for cows that had DME in every milking. The regression analysis revealed a significant negative association between DME%CAT and 10YIELD (Table 1). Cows with 0-10% DME had the highest least-square mean (LSM) for 10YIELD (426.8 kg), while cows with 91-100% DME had the lowest (338.9 kg), representing a difference of 87.9 kg over 10 days. This negative association was consistent across all farms, lactation, and stages of lactation, though the magnitude of the effect was modified by these factors. These findings provide evidence that the milk yield reduction associated with a DME event is not recovered in subsequent milkings and accumulates over time, supporting previous observational studies (Erskine et al., 2019). The results contradict some controlled experiments that found no yield difference between treatment groups with higher DME proportions with groups with lower DME proportions (Bruckmaier & Blum, 1996; Kaskous & Bruckmaier, 2011; Tuor et al., 2023) , a discrepancy potentially attributable to differences in study design, herd size, production level, and milking frequency.

Table 1. Multiple regression analysis of total milk yield over 10 days for 8447 cows from 4 commercial dairy farms using categories of percentage of DME events over 10 days.

Variable	Estimate	SE	***P*-value**	LSM	LSM 95% CI	Overall *P*-value
Intercept	425.2	3.2	**<0.001**			
Categories of percentage of DME events over 10 days						<0.001
0-10% (referent)	-	-	**-**	426.8	421.3-432.3	
11-20%	-11.1	7.6	**0.144**	415.6	409.6-421.6	
21-30%	-11.7	9.5	**0.219**	411.5	404.3-418.7	
31-40%	-17.4	10.3	**0.093**	403.1	395.3-411.0	
41-50%	-8.6	12.0	**0.478**	393.4	384.7-402.1	
51-60%	-31.4	13.7	**0.022**	382.2	373.3-391.1	
61-70%	-22.3	12.4	**0.071**	385.4	376.8-393.9	
71-80%	-52.2	12.2	**<0.001**	381.94	372.2-391.7	
81-90%	-27.7	11.4	**0.015**	364.24	354.1-374.3	
91-100%	-53.0	11.1	**<0.001**	338.9	328.8-349.0	
Farm						<0.001
Farm A (referent)	-	-	**-**	411.5	407.9-415.1	
Farm B	5.8	4.5	**0.194**	414.5	406.6-422.3	
Farm C	-21.9	3.3	**<0.001**	396.7	394.0-399.4	
Farm D	-67.8	9.8	**<0.001**	338.6	333.0-344.1	

Lactation						<0.001
1st (referent)	-	-	-	354.9	351.6-358.4	
2nd	81.6	3.5	**<0.001**	410.5	406.9-414.1	
3rd and greater	100.3	3.0	**<0.001**	405.5	401.8-409.1	
Stage of lactation curve						<0.001
21 ≤ 60 DIM	-	-	-	415.8	410.7-421.0	
61-100 DIM	-5.7	3.6	**0.119**	421.1	416.2-426.0	
101-150 DIM	-35.7	3.7	**<0.001**	409.4	404.9-413.8	
151-250 DIM	-62.0	3.6	**<0.001**	376.7	373.1-380.3	
>250 DIM	-87.0	4.8	**<0.001**	328.5	324.2-332.8	
DME%CAT x FARM						<0.001
DME%CAT x LACT						<0.001
DME%CAT x SLCURVE						<0.001

Conclusion

A higher frequency of delayed milk ejection events is significantly associated with a reduction in accrued milk yield over time in high-producing, 3X-milked herds. This suggests that DME can have a sustained negative economic impact. Since proper premilking procedures are critical for preventing DME, their role in maximizing yield should be emphasized as a key component for optimal udder health and farm profitability.

References

Bruckmaier, R. M., & Blum, J. W. (1996). Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation. *Journal of Dairy Research*, *63*(2), 201–208. https://doi.org/10.1017/s0022029900031708

Bruckmaier, R. M., & Blum, J. W. (1998). Oxytocin Release and Milk Removal in Ruminants. *Journal of Dairy Science*, *81*(4), 939–949. https://doi.org/10.3168/jds.S0022-0302(98)75654-1

Dahl, M., & Wieland, M. (2025). Influence of delayed milk ejection on mammary gland health and milking performance in dairy cows: A systematic review and meta-analysis. *Research in Veterinary Science*, *183*. https://doi.org/10.1016/j.rvsc.2024.105510

Erskine, R. J., Norby, B., Neuder, L. M., & Thomson, R. S. (2019). Decreased milk yield is associated with delayed milk ejection. *Journal of Dairy Science*, *102*(7), 6477–6484. https://doi.org/10.3168/jds.2018-16219

Fernandes, S., Pereira, G., & Bexiga, R. (2023). Bimodal milk flow and overmilking in dairy cattle: risk factors and consequences. *Animal*, *17*(3). https://doi.org/10.1016/j.animal.2023.100716

Kaskous, S., & Bruckmaier, R. M. (2011). Best combination of pre-stimulation and latency period duration before cluster attachment for efficient oxytocin release and milk ejection in cows with low to high udder-filling levels. *Journal of Dairy Research*, *78*(1), 97–104. https://doi.org/10.1017/S0022029910000816

Moore-Foster, R., Norby, B., Schewe, R. L., Thomson, R., Bartlett, P. C., & Erskine, R. J. (2019). Herd-level variables associated with delayed milk ejection in Michigan dairy herds. *Journal of Dairy Science*, *102*(1), 696–705. https://doi.org/10.3168/jds.2018-14561

Tuor, M., Wellnitz, O., & Bruckmaier, R. M. (2023). The interplay of continuous milk ejection and milking system with and without prestimulation at different vacuum settings. *Journal of Dairy Science*, *106*(5), 3615–3624. https://doi.org/10.3168/jds.2022-22661

Accelerating Diagnosis: A Culture-Independent Approach to Pathogen and Resistance Detection in Bovine Mastitis

Crystal Chapagain[1], Abdolrahman Khezri[1], Jawad Ali[1], Asal Ahmadi[1], Håvard Nørstebø[2], Marit Smistad[2], Liv Sølverød[2], and Rafi Ahmad[1*]

[1] University of Inland Norway, Hamar, Norway
[2]TINE SA, Oslo, Norway
***Corresponding author's email: rafi.ahmad@inn.no**

Introduction

Mastitis is the second-costliest dairy cattle disease worldwide, with an estimated annual cost of US$21 billion. (Rasmussen et al., 2024). This udder inflammation is caused by bacteria in 95% of cases and is treated with antibiotics. Detecting pathogens and testing antibiotic susceptibility in routine practice is currently culture-based, taking 3 to 5 days and often demonstrating limited sensitivity (Imam et al., 2024). Approximately 70% of the total antibiotics are used in animals (Tiseo et al., 2020). Rapid and accurate identification of causative pathogens is crucial to prevent the imprudent use of antibiotics, helping to curb the spread of antimicrobial resistance (AMR) and reduce economic losses. Long-read metagenomic sequencing offers a strong alternative for fast, accurate detection of pathogens and associated AMR, supporting more informed and targeted treatment. Many studies have demonstrated the effectiveness of this approach using Oxford nanopore sequencing technology in human biological samples (Ali et al., 2024; Bellankimath et al., 2024; Li et al., 2025). However, this approach has not been tested on bovine mastitis milk samples. The complex milk matrix, low bacterial levels, and high somatic cell counts in mastitis-affected milk often make this method difficult. This study aimed to develop a technique that can remove the

milk matrix, deplete or minimize host DNA, and enrich bacterial DNA to enable a rapid, culture- and amplification-free method for detecting pathogens and AMR genes from mastitis milk samples using real-time nanopore sequencing.

Material & methods

Here, we tested 34 bovine milk samples (28 with mastitis and 6 without). In the first study, we evaluated four different commercial kits (Qiagen's DNeasy R© PowerFood R© Microbial, Norgen's Milk Bacterial DNA Isolation, and Molzym's MolYsisTM Plus and Complete5) in combination with filtration, low-speed centrifugation, nuclease, and 10% bile extract from male bovine (Ox bile). In the follow-up study, additional optimizations were performed for removing sample matrix components, including combining centrifugation, gradient centrifugation, and fat fraction treatment with Tween 20 and citric acid. Subsequently, four DNA extraction kits (Blood and Tissue, Molysis Complete5, HostZero, and SPINeasy Host depletion) were evaluated for their ability to remove host DNA and enrich bacterial DNA. In both studies, the extracted DNA was quantified, checked for the presence or absence of host and pathogen DNA using PCR and qPCR, and sequenced using MinION nanopore sequencing. Real-time bioinformatics analysis was conducted for taxonomic classification and detection of antimicrobial resistance genes.

Results & Discussion

In the first study, kits specifically designed for bacterial DNA isolation from food and dairy matrices failed to deplete or reduce host DNA. The MolYsis Complete5 kit, combined with additional micrococcal nuclease and 10% ox bile, achieved up to 80% host DNA depletion, 100% detection sensitivity, and 92.3% specificity for identifying *S. aureus*, *E. coli*, and *S. dysgalactiae*. Major AMR genes, including tet(38), fosB-Saur, and blaZ, were detected within 5–9 hours of sample collection (Figure 1). The follow-up optimization study showed that simple centrifugation effectively concentrates bacterial cells without the need for additional chemical treatments. To further improve microbial DNA recovery, we compared several commercially available host depletion kits with the modified MolYsis Complete5 protocol. Among these, the HostZero kit consistently produced higher DNA yields, better DNA integrity, and more effective host DNA depletion. Using the optimized workflow, we successfully identified both Gram-positive and Gram-negative mastitis pathogens, along with their AMR genes, using PCR barcoding and nanopore sequencing. Since the standard MinION library preparation typically requires approximately 200 ng of DNA per barcode, which can be challenging to obtain in low-bacterial-load samples following host depletion, the PCR barcoding kit, which needs only 1-5 ng of DNA input, offers a practical solution for low-yield samples.

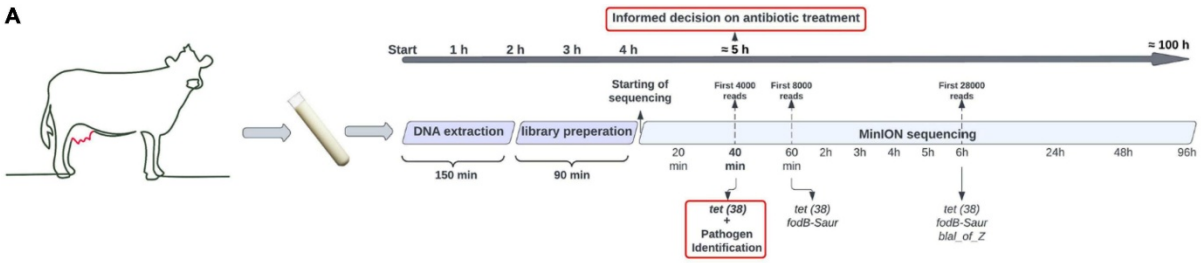

Figure 3. Timing of experiment and time/reads required for pathogen and AMR gene detection following FAST base calling and using real-time data analyses

Conclusion

We implemented an effective method (sensitivity of 100% and specificity of 92.3%) for host DNA removal and bacterial DNA enrichment (both gram-negative and positive) directly from bovine mastitis milk. To the best of our knowledge, this is the first culture- and amplification-independent study using nanopore-based metagenomic sequencing for real-time detection of the pathogen (within 5 hours) and the AMR profile (within 5–9 hours), in mastitis milk samples. The centrifugation-based bacterial enrichment, selective host DNA depletion, and Oxford Nanopore long-read sequencing workflow offers a quick, accurate, and culture-free method for detecting pathogens and AMR genes directly from mastitis milk. Compared to traditional diagnostics, this method provides faster results, higher sensitivity, and better genomic detail, supporting precise antimicrobial use and improved mastitis management. Future large-scale validation across different pathogens and sample types will confirm its suitability for on-farm or point-of-care diagnostics, enabling timely treatment and antimicrobial stewardship in dairy herds.

References

Ali, J., Johansen, W., & Ahmad, R. (2024). Short turnaround time of seven to nine hours from sample collection until informed decision for sepsis treatment using nanopore sequencing. *Scientific Reports*, *14*(1), 6534. https://doi.org/10.1038/s41598-024-55635-z

Bellankimath, A. B., Chapagain, C., Branders, S., Ali, J., Wilson, R. C., Johansen, T. E. B., & Ahmad, R. (2024). Culture and amplification-free nanopore sequencing for rapid detection of pathogens and antimicrobial resistance genes from urine. *European Journal of Clinical Microbiology & Infectious Diseases*, *43*(11), 2177–2190. https://doi.org/10.1007/s10096-024-04929-1

Imam, T., Horsman, S., Wood, B., Grewar, J. D., Langhorne, C., Price, R., Wood, C., Henning, J., & Gibson, J. S. (2024). Assessment of sensitivity and specificity of bacterial culture and the VetMAX™ MastiType Multi Kit in detecting Streptococcus uberis and Escherichia coli in milk samples from dairy cows with clinical mastitis in subtropical Australia. *Preventive Veterinary Medicine*, *233*, 106358. https://doi.org/10.1016/j.prevetmed.2024.106358

Li, G., Sun, H., Ye, Y., Chen, L., Zhang, W., Yu, S., Li, Q., & Fan, L. (2025). Clinical utility of nanopore-targeted sequencing for diagnosing and treating pulmonary infectious diseases from bronchoalveolar lavage fluid. *Frontiers in Cellular and Infection Microbiology*, *15*, 1469440. https://doi.org/10.3389/fcimb.2025.1469440

Rasmussen, P., Barkema, H. W., Osei, P. P., Taylor, J., Shaw, A. P., Conrady, B., Chaters, G., Muñoz, V., Hall, D. C., Apenteng, O. O., Rushton, J., & Torgerson, P. R. (2024). Global losses due to dairy cattle diseases: A comorbidity-adjusted economic analysis. *Journal of Dairy Science*, *107*(9), 6945–6970. https://doi.org/10.3168/jds.2023-24626

Tiseo, K., Huber, L., Gilbert, M., Robinson, T. P., & Van Boeckel, T. P. (2020). Global Trends in Antimicrobial Use in Food Animals from 2017 to 2030. *Antibiotics*, *9*(12), 918. https://doi.org/10.3390/antibiotics9120918

Matching milking machine settings to milk flow in a grazing system

Martin Browne*[1], Pablo Silva Boloña[1] and John Upton[1]

[1] Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

***Corresponding author's email: martin.browne@teagasc.ie**

Introduction

Modern milking management aims to achieve efficient and gentle milk removal to a user defined end-point. Because milk flow changes dynamically throughout milking and influences vacuum exposure at the teat, this study evaluated whether adjusting automatic cluster removal (ACR) thresholds and pulsation settings according to milk flow profiles could improve milking efficiency without compromising teat condition in a grazing system.

Previous research demonstrated the ability to curtail the low flow period towards the end of milking, where vacuum load on the teat is greatest, without impacting milk yield or somatic cell count (SCC) (Ginsberg et al., 2018; Upton et al., 2023). This research examined if more ambitious pulsation settings could be applied during the peak flow period of milking in conjunction with higher ACR flow rate threshold to further improve milking efficiency without adversely impacting teat condition. The work was conducted across lactation in a pasture-based system characterised by lower milk yields and distinct milk flow profiles relative to intensive indoor systems.

Material & methods

Two studies were conducted on a 46-unit Dairymaster rotary parlour at Moorepark dairy research farm (Ireland) during 2023 using two ACR flow rate thresholds (0.2 & 0.8 kg/min) together with static or dynamic pulsator settings giving a total of four treatment combinations. Dynamic pulsation increased the open phase of pulsation ratio from 63 to 73% when milk flow exceeded 2 kg/min in the first study (Upton et al., 2025). Average daily milk yield at the start of the first study was 23.5 kg/min. The second study (Upton et al, in press), using cows from the same spring calving pasture-based herd, was conducted later in lactation when average daily milk yield was 16.4 kg/min. It further

categorised cows into high and low milk flow rate groups with the flow rate threshold for the dynamic pulsation setting lowered to 1.5 kg/ min.
A full crossover design was implemented where cows transitioned through each treatment in random sequence, spending a fortnight on each, resulting in two eight-week studies. All procedures were approved by the Teagasc Animal Ethics Committee (TAEC2021-321) and conducted in accordance with relevant national animal welfare legislation.
Milk yield, milking duration and milk flow were recorded at each milking. Weekly milk composition and SCC were analysed, teat condition was assessed fortnightly, and vacuum at the teat end and base was recorded on a representative subset using VaDia loggers. Data were analysed using mixed models with repeated measures.

Results & Discussion

Milk yield, total solids yield, and $\log_{10}$ SCC were not affected by treatment. Increasing the ACR flow-rate threshold from 0.2 to 0.8 kg/min reduced milking duration by 15% early in lactation, rising to 25% later in lactation. In the second study, the proportional reduction in milking duration between ACR settings was greater for low-flow cows than for high-flow cows. These findings indicate that increasing the cluster removal threshold is most impactful under low udder-fill conditions.
Little difference was found in milking time through the institution of dynamic pulsation settings. However, in both studies, cows spent less than a third of total milking time, on average, above the flow rate threshold for increased open phase pulsation settings. A small but significant increase in average milk flow ($P = 0.02$) was observed for dynamic compared with static pulsation when the ACR threshold was 0.8 kg/min during AM milkings, but no effect was detected in PM milkings, where the proportion of high-flow time was lower.
In production systems with higher milk yield and greater udder fill, the proportion of milking time above the activation threshold for dynamic pulsation would likely increase. Under such conditions, the impact of dynamic pulsation on milking duration may be greater.
Vacuum measurements obtained using VaDia loggers indicated lower short milk tube vacuum during the main milking period for treatments combining dynamic pulsation with the higher ACR threshold. However, dynamic pulsation did not improve post-milking teat condition, whereas increasing the ACR switch-point to 0.8 kg/min reduced odds of teat-barrel congestion. Low-flow cows exhibited a higher prevalence of teat congestion overall.

Conclusion

Increasing the ACR switch-point from 0.2 to 0.8 kg/min reduced milking duration by 15–25% and reduced teat-barrel congestion without affecting milk yield or SCC. Dynamic pulsation had limited impact on milking duration and teat condition but modified vacuum dynamics during milking. Greater proportional reductions in milking duration were observed in low-flow cows and later in lactation.

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References

Ginsberg, R., Dzidic, A., Rasmussen, M., Poulet J., Manninen, E., Sigurdsson, S., Tančin, V. & Bruckmaier, R. (2018). Teat-cup and cluster removal strategies for cattle and small ruminants, Review and recommendations. *Bulletin of the IDF No. 491/ 2018*. International Dairy Federation.

Upton, J., Browne, M. & Silva Boloña, P. (2023). Effect of milk flow-rate switch-point settings on milking duration and udder health throughout lactation. *Journal of Dairy Science, 106 (2023),* 8861-8870. https://doi.org/10.3168/jds.2023-23559

Upton, J., Browne, M. & Silva Boloña, P. (2025). Effect of dynamic pulsation and milk flow rate switch-point settings on milking duration and postmilking teat condition. *Journal of Dairy Science, 108 (2025),* 2632-2641. https://doi.org/10.3168/jds.2024-25888

Upton, J., Browne, M. & Silva Boloña, P. (in press). Differential responses of low- and high-flow dairy cows to automatic cluster removal and dynamic pulsation settings. *Journal of Dairy Science*.

Effect of cow-calf contact on udder emptying in dairy cows milked in a robotic milking unit

Sara Almlöf1*, Sabine Ferneborg2, Sigrid Agenäs1
1Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Sweden.
2Faculty of Biosciences, Norwegian University of Life Sciences, Norway.
***Corresponding author's email: sara.almlof@slu.se**

Introduction

There is an increasing interest worldwide in keeping machine milked dairy cows together with their calves for an extended period of time in different types of cow-calf contact (CCC) systems (1). When CCC is combined with automatic milking systems (AMS), problems with incomplete milkings occur. This may be caused by a variation in udder fill at milking, because of calves suckling, or that oxytocin released in response to the stimulation in the milking unit (MU) is significantly lower than levels that occur when cow and calf are interacting and that milk ejection therefore fails in the MU. However, methodology for sampling plasma oxytocin in CCC cows in AMS is not available and research therefore has to rely on indirect methods.

This study aimed to investigate if the presence of calves or the suckling in a CCC management system affects udder emptying in AMS and also to investigate if fat content in post-milking strip milk and the proportion of residual milk are correlated in CCC cows. The hypothesis was that the fat content of strip milk samples after machine milking can be used as an indicator of the degree of udder emptying and thereby be useful as an on-farm tool for evaluating milking success in CCC systems.

Material & methods

A total of 38 cows (12 Swedish Holstein, 26 Swedish Red) were divided into two different treatments, CCC (cow-calf contact) or CONV (conventional). The CCC cows had access

to their calves in a contact area built into the Voluntary Milking System (DeLaval VMS, International AB, Tumba, Sweden; VMS) with semi-controlled feed-first in a cow-driven traffic system (2). They could access the contact area any time of the day for 85±16 days and then underwent fenceline separation with part-time nursing access for 10 days, followed by 7 days with fenceline without any nursing access before total separation. Cows were milked in AMS, DeLaval VMS Classic MU and strip milk samples were collected by hand stripping from each quarter after machine milking. Residual milk was quantified using intramuscular injection of oxytocin, after the strip milk sampling and waiting 3 minutes before attaching the MU teat cups and milk again. Sampling was performed during three occasions: during full contact, part-time fenceline and two weeks after total separation. Data was analyzed with SAS software (SAS Enterprise guide 8.3) using mixed procedure (PROC MIXED) with a model including the fixed effects of sampling occasion, treatment (CCC, CONV), parity (1, 2, >2), breed (SH, SR), strip milk fat content and included a random statement for the repeated measures on each cow using a autoregressive covariance structure. The Tukey-Kramer adjustment was used to account for multiple comparisons. Quadratic regression analysis was used to test correlation between fat content in post-milking strip milk and proportion of residual milk. Proportion of residual milk was calculated as amount of residual milk divided by total milk yield and ln transformed before statistical analysis due to non-normal distribution.

Results & Discussion

The fat content in strip milk was lower for CCC cows than CONV ($P<0.001$). The proportion of residual milk in CCC cows differed between sampling occasion before and after total separation ($P<0.05$). There was a considerable variation in proportion of residual milk within all sampling occasions. A higher fat content in post-milking strip milk was associated with a lower proportion of residual milk ($P<0.001$). A lower proportion of residual milk after total separation indicates a more complete udder emptying. Quadratic regression analysis across the three sampling occasions showed a correlation between post-milking fat content in strip milk and proportion of residual milk, but more data is needed to ensure sufficient sensitivity and specificity for use as an on-farm tool. Persisting variation between cows in residual milk proportion indicates that incomplete milkings remains in some cows after separation from the calf and more research is needed to solve impaired milk ejection when CCC is combined with AMS. To clarify the role of udder fill at milking for successful milk ejection and milk removal in CCC cows, research data from cows milked at different milking and suckling-to-milking intervals is needed and we are currently running a trial with CCC and CONV cows randomized into 4- or 10- hours milking permission. In this ongoing trial position data combined with video analysis will be used to estimate last suckling prior to when cows visit the MU.

Conclusion

In conclusion, udder emptying during machine milking was less complete during cow-calf contact than after separation in most cows. Lower fat content in strip milk after machine milking was associated with higher proportion of residual milk. Further research is planned to confirm the use of post-milking fat content in strip milk as an indicator of the degree of udder emptying at commercial farms. Perhaps it could be used as a practical on-farm tool for evaluating milking success and detecting cows that may struggle with impaired milk ejection.

References

Eriksson H, Fall N, Ivemeyer S, Knierim U, Simantke C, Fuerst-Waltl B, Winckler C, Weissensteiner R, Pomiès D, Martin B, Michaud A, Priolo A, Caccamo M, Sakowski T, Stachelek M, Spengler Neff A, Bieber A, Schneider C, Alvåsen K. Strategies for keeping dairy cows and calves together - a cross-sectional survey study. Animal. 2022 Sep;16(9):100624. https://doi.org/10.1016/j.animal.2022.100624

Wegner C, Ternman E. 2023. Lying behaviour of lactating dairy cows in a cow-calf contact freestall system. Applied Animal Behaviour Science. Volume 259, 2023, 105851. ISSN 0168-1591. https://doi.org/10.1016/j.applanim.2023.105851

Evidence-Based Ethnoveterinary Interventions for Bovine Mastitis and Other Ailments

Dutta P.[1], Ponnanna N.M. [1], Harikumar A.V. [1], Shroff S[1], Dave K.M. [1], Punniamurthy N. [1], Gupta R.O. [1], Daniel N.D. [2], and Shah Meenesh[1]
[1]National Dairy Development Board, Gujarat, India; [2] International Dairy Federation
***Corresponding author's email: pdutta@nddb.coop**

Introduction

Mastitis is a major economic disease in dairy production, reducing milk yield and quality. Subclinical mastitis causes most losses, while clinical cases often lead to antibiotic use, contributing to antimicrobial resistance (AMR)1, higher costs, milk withdrawal losses, and residue risks. To address this, a structured programme across Milk Unions and Producer Companies in India promotes oral Tri Sodium Citrate (TSC) for management of subclinical mastitis2 (SCM) and standardized Ethnoveterinary Medicines (EVM) for common bovine ailments including mastitis.

Material & methods

A structured, multi-institutional programme was implemented across 12 Milk Unions and Producer Companies within India's cooperative dairy network to promote non-antibiotic interventions for mastitis and other common bovine ailments. The programme integrated oral trisodium citrate (TSC) for the management of subclinical mastitis (SCM) and standardized ethnoveterinary medicines (EVM) for mastitis and other common bovine ailments encountered in dairy cattle.

Capacity building of field personnel was conducted through a cascaded training model involving 1,449 veterinarians and 13,181 animal health personnel to ensure standardized implementation, diagnosis and case documentation across participating Milk Unions and Producer Companies (MU/PCs). Regular farmer awareness on clean milk production, SCM detection, EVM propagation and AMR were conducted across participating MU/PCs. Udder health surveillance was undertaken through large-scale screening using the California Mastitis Test (CMT) on pooled milk samples collected at the Dairy Cooperative Society (DCS) level. Farmers whose pooled milk samples tested positive were subsequently traced back to their households, where individual lactating

animals were screened using CMT to identify SCM-positivity. Animals testing positive for SCM were administered an oral regimen of TSC at a rate of 10 g per day for ten consecutive days given by mixing with drinking water or feed. Animals remaining CMT-positive on subsequent testing received an additional 10-day TSC regimen. Representative milk samples from clinical and subclinical mastitis were subjected to bacteriological examination and antibiotic sensitivity testing under a One Health framework.

Common bovine ailments including Clinical Mastitis were diagnosed by the veterinarians based on clinical signs. Ailment management outcomes were also documented based on observable clinical recovery indicators, including resolution of clinical signs and improvement of milk yield toward normal levels in lactating animals. Details were captured through a digital reporting system by the veterinarians to enable centralized monitoring and programme assessment.

	QR code linking to a video demonstrating the ethnoveterinary management of bovine mastitis

Extension materials on EVM, including booklets, trifolds, posters and videos have been developed in 11 Indian languages along with English to support wider outreach at the community and farmer levels. In addition, dedicated EVM production units have been established within selected Milk Unions (MUs) to ensure the standardized preparation and large-scale availability of ready-to-use EVM formulations.

Results & Discussion

The programme has achieved significant outcomes across 12 MU/PCs. To validate and demonstrate the efficacy of EVM at the DCS level, 803 demonstration plots have been established. Large-scale screening through CMT covered 2.15 million pooled milk samples (Figure 1), with a decline in the percentage of positive samples observed from 2017–18 to 2025–26, indicating improved udder health management.

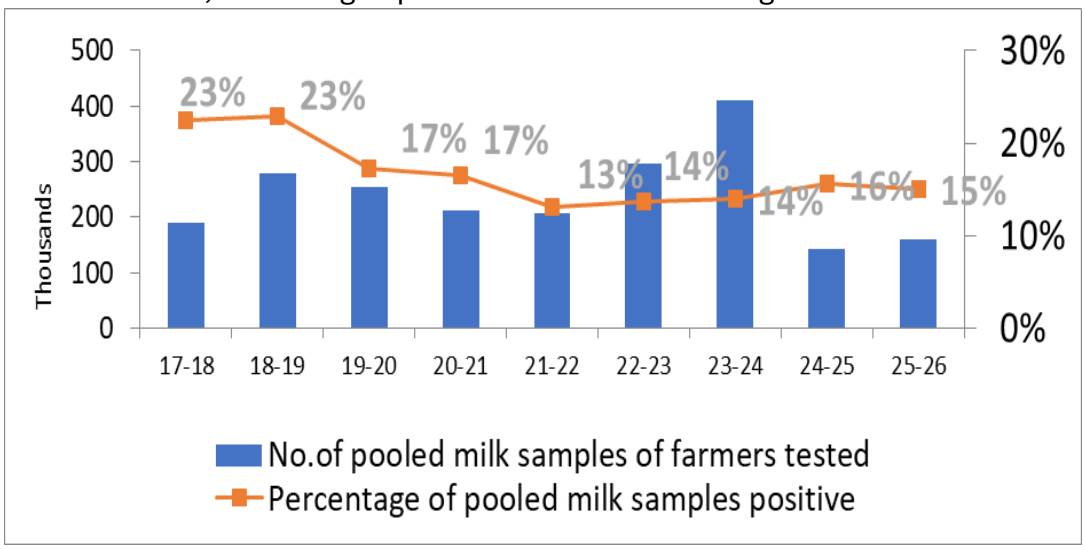

Figure 4 Year-wise number of pooled milk samples tested and percentage of positive samples (2017–18 to 2025–26).

Since 2017–18, a total of 1.27 million cases of common bovine ailments including mastitis, have been managed using EVM alone, where resolution of clinical signs and improvement of milk yield toward normal levels in lactating animals was observed in 80% of the cases after EVM intervention. Production of over 9 million EVM doses through Union-level EVM plants located across diverse regions of India ensures scalability, decentralized production, and long-term operational sustainability.

Through the management of SCM with TSC administration, extensive promotion of EVM for bovine ailments, and farmer awareness on judicious antibiotic use, one MU has reported progressive reductions in antibiotic usage (Figure 2).

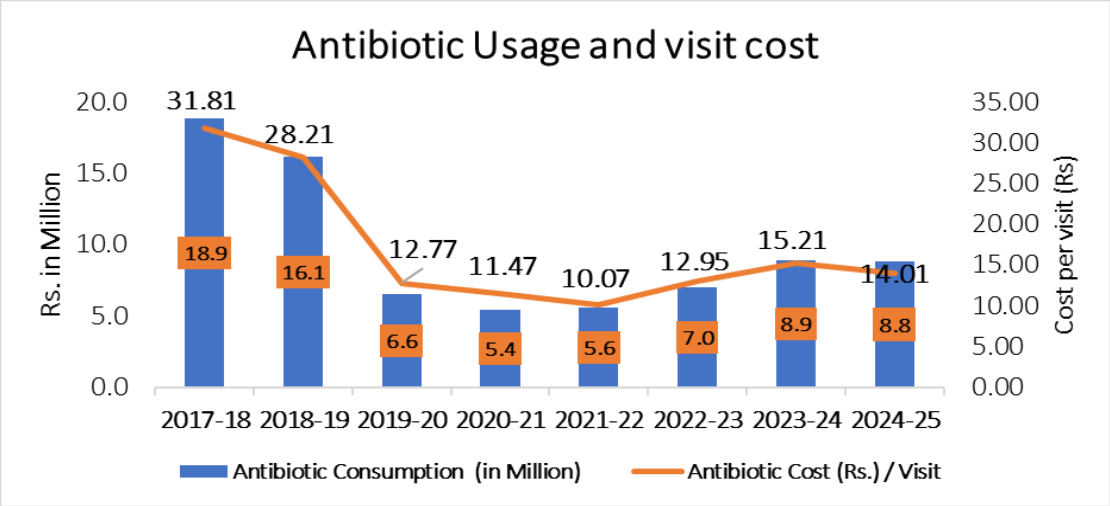

Figure 5 Annual antibiotic consumption (₹ million) and average antibiotic cost per veterinary visit (₹/visit) in a project area, 2017–18 to 2024–25.

Conclusion

This programme demonstrates the feasibility of integrating standardized ethnoveterinary practices and non-antibiotic interventions within cooperative dairy systems as part of a large-scale extension initiative supporting antimicrobial stewardship. The approach highlights how capacity building, surveillance, and farmer outreach can facilitate the adoption of alternative animal health management practices while contributing to reduced reliance on antibiotics. Such models provide a scalable framework for strengthening antimicrobial stewardship and promoting sustainable udder health management in smallholder-dominated dairy systems.

References

Maksimović, Z., Čengić, B., Ćutuk, A., & Maksimović, A. (2024). Antimicrobial Resistance of Cattle Mastitis-Causing Bacteria: How to Treat? In Veterinary Medicine and Science. IntechOpen.

Dutta, P., Harikumar, A. V., Patel, S. B., Patel, N. A., Patel, A. S., & Sharma, G. K. (2017). Prospects of controlling sub-clinical mastitis in cattle and buffaloes through the use of trisodium citrate. Indian Dairyman, 69(11), 62–65

Using milking technology data for new insights: Identification of hidden udder health patterns using sensor data

[1,4]Muhammad Ikhsan Sani, [1]Mariska van der Voort, [3]Ilka Christine Klaas, [2]Bedir Tekinerdogan and [1]Henk Hogeveen

[1]Business Economics Group, Wageningen University and Research, Wageningen 6706 KN, The Netherlands

[2]Information Technology Group, Wageningen University and Research, Wageningen 6706 KN, The Netherlands

[3]DeLaval International AB, Gustaf De Lavals väg 15, 147 21 Tumba, Sweden

[4]Computer Technology Study Program, School of Applied Science, Telkom University, Bandung 40257, Indonesia

***Corresponding author's email: ikhsan.sani@wur.nl**

Introduction

Mastitis is widely recognized as one of the costly production disease in dairy farming, both in terms of prevalence and economic impact (Hogeveen *et al.*, 2011).. Subclinical mastitis is commonly indicated by elevated somatic cell count (SCC) in milk (Kebede & Tilahun, 2023). Recent work shows that SCC behaviour over time contains informative patterns and SCC trajectories can reflect different udder health condition (Deng *et al.*, 2021, Bonestroo *et al.*, 2022). However, SCC patterns are often defined by expert or manual interpretation. Such approaches limits scalability and may introduce subjective bias.

Automatic milking (AMS) systems have generated large volumes of routinely collected cow-level sensor data for many years. Despite this availability, the use of SCC data based on on-line sensor systems is underexploited. SCC patterns are still mainly evaluated using threshold-based indicators rather than trajectory-based analysis. Machine learning (ML) has been increasingly applied in precision dairy farming targeting for mastitis detection (Neethirajan, 2020, Bobbo *et al.*, 2021). Unsupervised ML can be

used to discover recurring structures in SCC trajectories without predefined health condition labels. This approach makes unsupervised ML suitable for automated and data-driven pattern discovery in routinely collected SCC sensor data. Therefore, this study aims to identify unique SCC patterns by exploring routinely collected data from Voluntary Milking Systems (VMS) by using unsupervised ML.

Material & methods

Data description and preparation

Anonymized data were obtained from 18 dairy farms from The Netherlands and Sweden equipped with DeLaval VMS (VMS series; DeLaval International AB, Tumba, Sweden) and the DeLaval Online Cell Counter (OCC). The raw dataset consisted of 72 variables and 7,352,877 milking records from 4,682 cows. The number of lactating cows of each farm ranged from 92 to 679 cows. The following variables were selected from the raw dataset: cow identification number, OCC-based SCC measurement, milking start and end timestamps.

Lactations were reconstructed from milking timestamps for each cow. A new lactation was defined when the interval between two consecutive milking records exceeded one day. Based on the identified lactation start date, days in milk (DIM) were calculated for all subsequent milking events within that lactation. Only full lactations that have 305 DIM were included to ensure comparable trajectory length across cows, following standard dairy performance evaluation practice (Knight, 2005, Sawa *et al.*, 2015).

SCC measurements were not available for every milking event. Therefore, SCC values were aggregated into daily mean SCC per cow. For each lactation, SCC features were calculated for capturing their trajectories, including average daily SCC, median, slope over DIM (indicating trend), coefficient of variation, peak count (number of times SCC exceeded 400,000 cells/mL), autocorrelation coefficient describing temporal dependency, and short term fluctuation measured as variation between consecutive daily SCC values (Fulcher *et al.*, 2013, Deng *et al.*, 2021). All relevant features were normalized using Python StandardScaler library to ensure that features with larger numerical ranges did not influence the clustering algorithm.

Data analysis

K-means clustering algorithm was applied to the normalized SCC feature matrix using the scikit-learn ML library (Hastie et al, 2009(Pedregosa *et al.*, 2011). The feature matrix consisted of cow–lactation observations as rows and extracted SCC trajectory features as columns. The clustering workflow included construction of the feature matrix, evaluation of candidate numbers of clusters (k), and selection of the optimal cluster number using the elbow method (k = 8). The final model was fitted using multiple centroid initializations (n_init = 2–10) to reduce sensitivity to random initialization.

Results & Discussion

Preliminary results showed that k-means clustering identified eight SCC trajectory clusters across the 305 DIM lactation period (Figure 1). The identified SCC trajectory types were compared with patterns reported by Deng et al. (2021). The clusters differed in baseline SCC level, variability, and temporal development. Clusters 4 and 7 show persistently low SCC values throughout lactation with minimal variability. Cluster 1 shows generally low SCC levels interrupted by occasional short-duration peaks exceeding the SCC threshold. Clusters 2 and 5 show SCC variability with repeated fluctuations across lactation. Cluster 6 shows consistently elevated SCC values across most of lactation. Cluster 8 shows high SCC levels during early lactation followed by a gradual decline toward low SCC values.

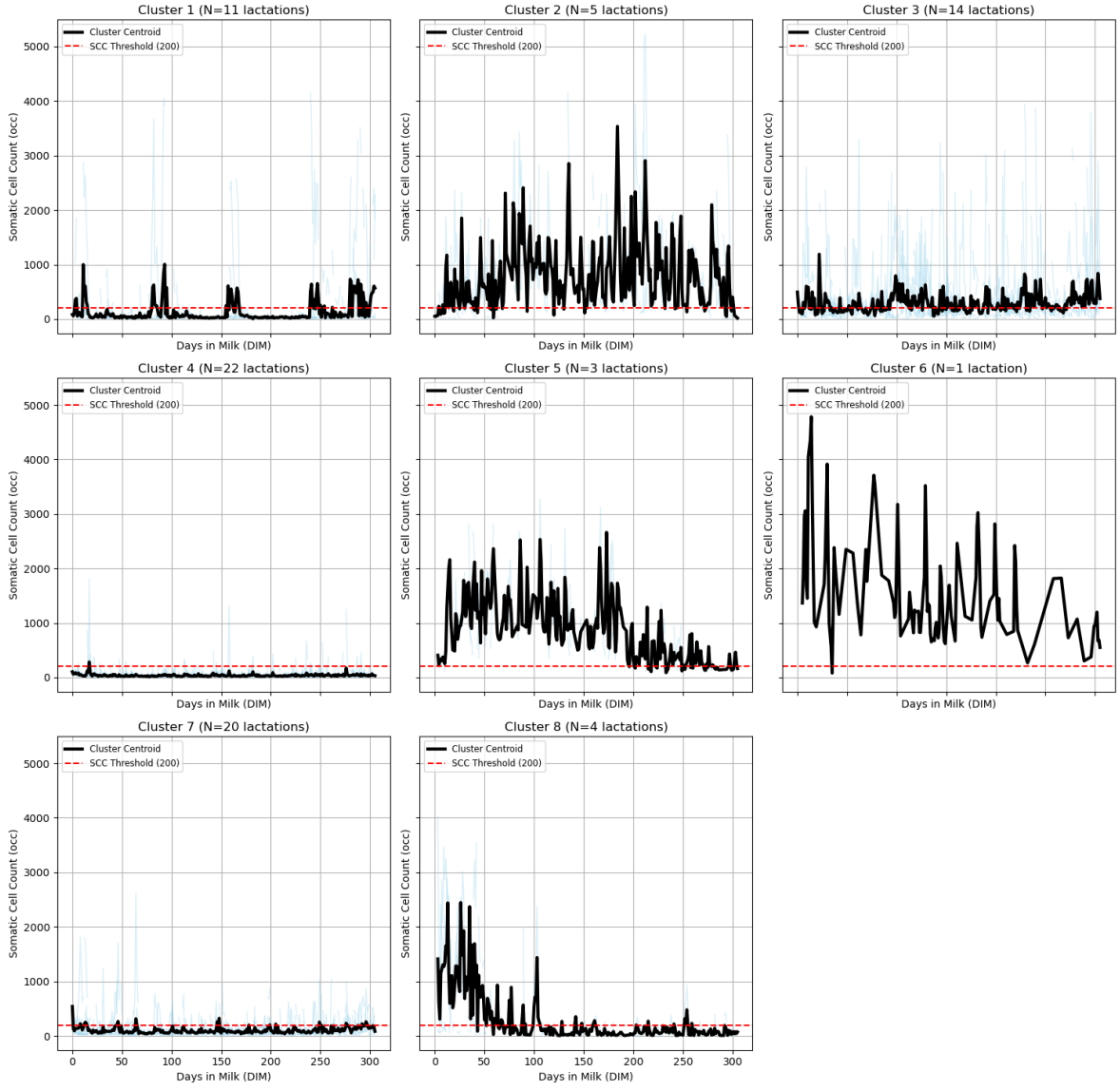

Figure 6: Identified SCC Patterns across clusters (DIM 0-305). Dash line represent SCC threshold 200,000 cells/mL

Conclusion

Unsupervised ML applied to VMS data enabled identification of distinct SCC trajectory clusters without predefined labels from expert. The identified clusters reveal recurring

structure in SCC trajectory and demonstrate that the patterns can be derived by machine from sensor data.

References

Bobbo, T., Biffani, S., Taccioli, C., Penasa, M., and Cassandro, M. (2021). Comparison of machine learning methods to predict udder health status based on somatic cell counts in dairy cows. *Scientific Reports* 11(1), 13642.

Bonestroo, J., Voort, M.V.D., Hogeveen, H., Emanuelson, U., Klaas, I.C., and Fall, N. (2022). Forecasting chronic mastitis using automatic milking system sensor data and gradient-boosting classifiers. *Computers and Electronics in Agriculture* 198(

Deng, Z., Lam, T., Hogeveen, H., and Koop, G. (2021). Regularly fluctuating somatic cell count pattern in dairy herds. *Journal of Dairy Science* 104(10), 11126-11134.

Fulcher, B.D., Little, M.A., and Jones, N.S. (2013). Highly comparative time-series analysis: The empirical structure of time series and their methods. *J R Soc Interface* 10(83), 20130048.

Hogeveen, H., Huijps, K., and Lam, T.J. (2011). Economic aspects of mastitis: New developments. *New Zealand Veterinary Journal* 59(1), 16-23.

Knight, C.H. (2005). Extended lactation: Turning theory into reality. *Advances in Dairy Technology* 17(113-123.

Neethirajan, S. (2020). The role of sensors, big data and machine learning in modern animal farming. *Sensing and Bio-Sensing Research* 29(

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, A., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dunbourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., and Duchesnay, E. (2011). Scikit-learn: Machine learning in python. *Journal of Machine Learning Research* 12(

Sawa, A., Krężel-Czopek, S., and Bogucki, M. (2015). Dry period length as related to milk yield and scc during the first month of subsequent lactation. *Annals of Animal Science* 15(1), 155-163.

Effect of preservation, temperature and storage on the detection of proteolytic *Pseudomonas* spp.

Alexandra Beckmann [1*], Karin Knappstein[1] and Gregor Fiedler[2]

[1] Institute of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany

[2] Institute of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Kiel, Germany

***Corresponding author's email: alexandra.beckmann@mri.bund.de**

Introduction

Pseudomonas spp. have been shown to cause milk spoilage due to the ability to produce proteolytic enzymes such as peptidases (Machado et al., 2017). Due to their heat resistance, it is important to minimize the bacterial counts of *Pseudomonas* spp. in raw milk during milk extraction, storage and transport. In summer 2024, a dairy company reported spoilage problems in ultra-high temperature (UHT) processing milk caused by heat-resistant peptidases (Machado et al., 2017). In this context, it was hypothesized that the detection of *Pseudomonas* spp. with proteolytic activity was insufficient by flow cytometry and that the preservation of bulk milk samples with acidiol could potentially result in a reduction of the total bacterial count (TBC). In order to accurately assess problems related to the microbiological quality in dairy farms, it is essential that relevant bacterial groups are appropriately detected in bulk tank milk. The present study therefore investigated the effect of preservation, temperature and storage duration on the detection (flow cytometry and standard plate count in comparison) of different strains of *Pseudomonas* in dependence of varying proteolytic activity.

Material & methods

The experiment was conducted in two periods (September and December 2024). Strains of *Pseudomonas* were obtained from the strain collection of the institute and mainly were isolated from raw milk samples in Germany (Gieschler-Lübbehüsen et al., 2025). The selection was based on abundance in raw milk and proteolytic activity level measured as proteolytic index (given in brackets) according to Hamdani et al. (2019): Very high to high: *P. proteolytica* L1-105 (2.05); *P. gessardii* L1-90 (1.75), Moderate: *P. gessardii* L1-89 (1.55); *P. fluorescens* L1-82 (1.26); *P. proteolytica* L1-106 (1.00), No detectable peptidase activity: *P. ludensis* L1-94; *P. fragi* L1-86. Additionally, the mesophilic species *P. aeruginosa* DSM 50071 was included.

Each isolate was streaked onto one Columbia blood agar plate containing 5% sheep blood and incubated at 30 °C for 24h. Afterwards one inoculating loop was transferred into 10 ml Caso bouillon and cultivated another 24h at 30 °C. All bacterial suspensions were transferred to raw milk with an initial TBC below 520 cfu/mL, in order to adjust the *Pseudomonas* cell density in the raw milk to a range of $10^4 – 10^5$ cfu/mL. Raw milk without inoculation was used as control milk.

The nine milk samples were divided into subsamples, stored at two different temperatures (6, 10 °C) and analyzed for TBC at 24h-intervals during storage periods of 0 to 72h. The TBC was analyzed using the reference colony count method (ISO 4833-1) and by flow cytometry using BactoScan FC (Foss Electric, Hillerød, Denmark). Results were expressed as individual bacterial count (IBC) and colony forming unit (cfu) per mL for flow cytometry and standard plate count, respectively. Both values were $\log_{10}$-transformed. The data were analyzed descriptively using SAS Version 9.4.

Results & Discussion

Bacterial counts of *Pseudomonas* in samples preserved with acidiol remained stable at 6 and 10 °C over 72h (Figure 1; $\log_{10}$ IBC/ml in period 1 shown). The average TBC for preserved *Pseudomonas* samples was 4.3 $\log_{10}$ IBC/mL and 4.6 $\log_{10}$ cfu/mL at 0h and 4.2 $\log_{10}$ IBC/mL and 4.4 $\log_{10}$ cfu/mL after 72h. For unpreserved samples an increase of psychrophilic *Pseudomonas* of 1.2 $\log_{10}$ IBC/mL and 1.9 $\log_{10}$ IBC/mL was already observed after 24h at 6°C and 10°C, respectively (Figure 1). As expected, there was no growth of the mesophilic *P. aeruginosa*.

In general, the increase of TBC at 10°C was higher than at 6°C and highlights the importance of cold temperatures during storage and milk transport. As the bacterial counts of *Pseudomonas* increased over 72h, short storage and transport durations of raw milk are also essential. Except for *P. aeruginosa*, the bacterial growth curves of *Pseudomonas* strains did not differ in dependence of different proteolytic activity levels (Figure 1).

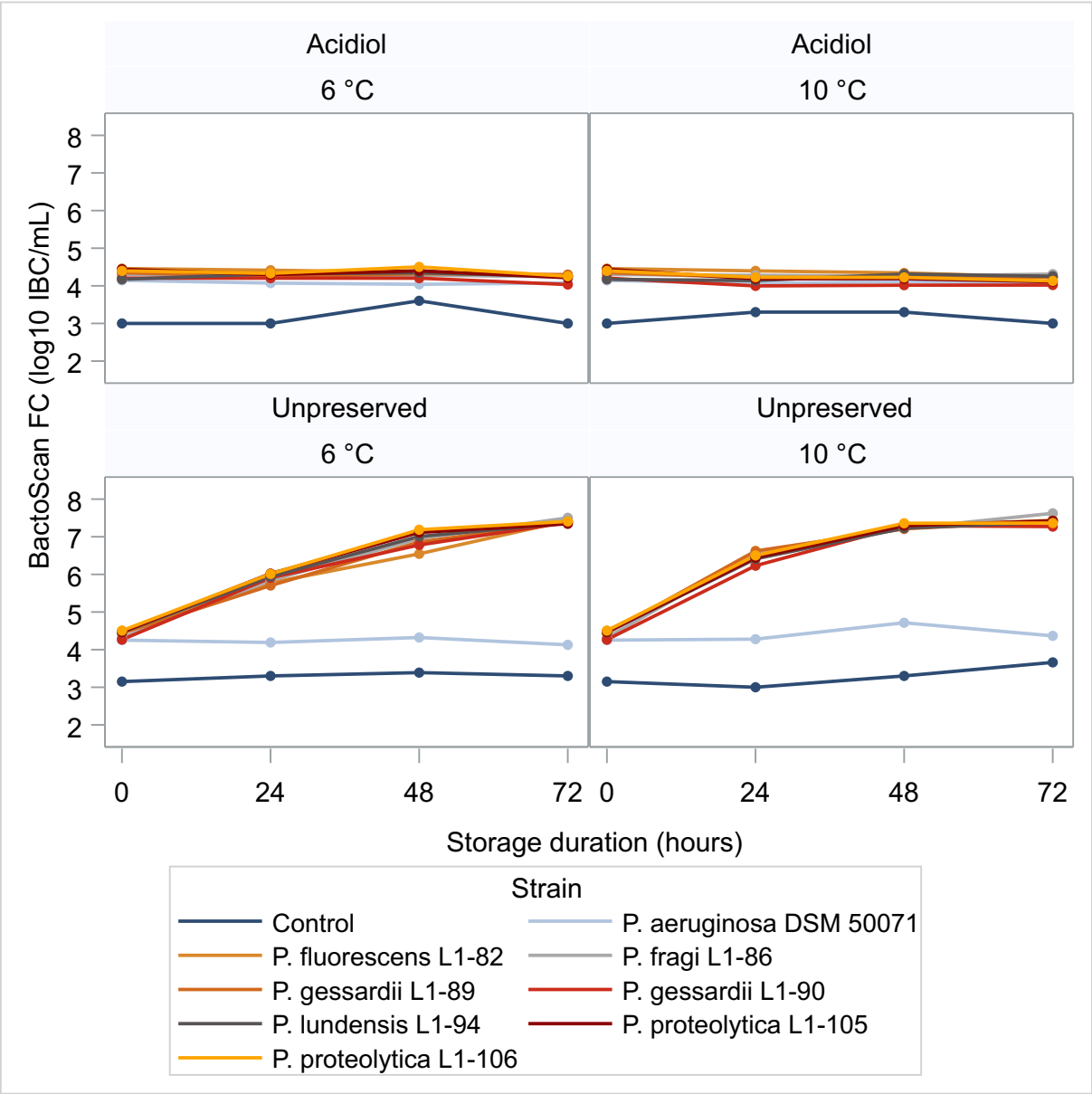

Figure 7. Growth curves in period 1 of different *Pseudomonas* strains with varying proteolytic activity (very high to high (red), moderate (orange), no detectable peptidase activity (grey)) stored at different temperatures (6, 10°C) with (Acidiol) and without preservation during 0 to 72h.

Although results provided insights into the growth curves of *Pseudomonas* strains with different proteolytic indices, the following factors should be considered when interpretating the results: Only eight strains of *Pseudomonas* were included in the experiment with relatively high initial counts. Furthermore, the culture consisted predominantly of one *Pseudomonas* species, with only minor presence of other microorganisms. Thus, further experiments should include more *Pseudomonas* strains with different initial bacterial counts used for inoculation of raw milk.

Conclusion

Stable *Pseudomonas* counts in milk samples preserved with acidiol were measured by flow cytometry. Therefore, it seems improbable that insufficient detection of poor quality milk with high counts of *Pseudomonas* spp. contributed to the spoilage problem in UHT milk. The observation of fast increase of these psychrophilic bacteria at storage temperatures of 6 and 10 °C underlines the necessity of aiming for very low initial bacterial counts by appropriate hygiene management. This also involves maintaining the cold chain and ensuring that raw milk is stored and transported for short periods prior to further processing.

References

Gieschler-Lübbehüsen, S., Kabisch, J., Hetzer, B., Franz, C. M., & Böhnlein, C. (2025). Risk of *Pseudomonas* spp. in raw milk: Biofilm formation and enhanced peptidase production. *International Dairy Journal*, *171*, 106375. https://doi.org/10.1016/j.idairyj.2025.106375

Hamdani, S., Asstiyani, N., Astriany, D., Singgih, M., & Ibrahim, S. (2019). Isolation and identification of proteolytic bacteria from pig sludge and protease activity determination. *IOP Conference Series: Earth and Environmental Science*, *230*, 12095. https://doi.org/10.1088/1755-1315/230/1/012095

International Organization for Standardization (2013). *ISO 4833-1:2013 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms:* Part 1: Colony count at 30 °C by the pour plate technique (ISO 4833-1).

Machado, S. G., Baglinière, F., Marchand, S., van Coillie, E., Vanetti, M. C. D., Block, J. de, & Heyndrickx, M. (2017). The Biodiversity of the Microbiota Producing Heat-Resistant Enzymes Responsible for Spoilage in Processed Bovine Milk and Dairy Products. *Frontiers in Microbiology*, *8*, 302. https://doi.org/10.3389/fmicb.2017.00302

Effect of Different Vacuum Settings on Cow Behavior During Milking

(Effect of vacuum on behavior)

Paola Bacigalupo Sanguesa[1*], Oskar Paulrud[2], Konstantinos Kakoulis[2], Doug Reinemann[3]

[1] Michigan State University, Lansing, USA
[2]Delaval, Thumba Sweeden
[3]University of Wisconsin, Madison, USA
***Corresponding author's email: paolabs@msu.edu**

Introduction

The goal of the milking process is to harvest as much milk in the most efficient manner as possible while ensuring the cows' comfort. Great progress is being achieved in the first 2 goals, however, the latter requires some consideration.

Bimodal milk letdown or delayed milk ejection can be observed in all herd types and sizes, with herd prevalences ranging from 0 to 86% (Edwards et al., 2014; Fernandes et al., 2023; Moore-Foster et al., 2019). Bimodal letdown can cause discomfort. During the periods of low milk flow, cows' teats are exposed to high milking vacuum (Browne et al., 2024) which increases the strain on the teats (Wieland et al., 2020). Cows express discomfort during milking by increased stepping and kicking behaviors, thus, they have been used to evaluate comfort (Browne et al., 2024).

Recently developed milking technology that adapts the vacuum to individual cow's milk flow might be an alternative to control this negative effect of bimodal letdown (Reinemann et al., 2021). Therefore, the main objective of this project is to evaluate the cow's behavior as a proxy from comfort during the milking process when exposed to different vacuum settings (constant vs flow adaptive vacuum).

Material & methods

We enrolled one farm equipped with a parlor with milking vacuum adjusting technology (Flow-Responsive Milking, DeLaval) and milking recording software (DelPro, DeLaval). Our experiment followed a completely randomized design, with the cow as the experimental unit. Our treatments consisted of a flow adjusted vacuum stetting with a

milking vacuum of 44 kPa during low to normal flow (<2kg/min) with vacuum of 49 kPa during peak flow (44-49V), constant milking vacuum at 49 kPa (49V), and the control was consistent vacuum at 44 kPa (CON). We randomly allocated the treatments to the milking stalls (double 15 parallel parlor) during a single milking. The treatments were applied after a period of mechanical stimulation provided by the liners. Using a modified milking cluster, we placed accelerometers data loggers in each milking unit to record activity during the milkings. From milking recording software, we collected individual cow information for the milking session when the experiment was conducted including animal number, parity, DIM, group, time of start milking session, stall number, and yield. Additionally, the software automatically assigns the cows' milk flow to 6 categories during a milking; we used the order of those categories to identify the presence of bimodal milk ejection. Next, we will use the accelerometer recordings to evaluate the cow's stepping behavior as a proxy of comfort during milking. We will match the data from accelerometers with the parlor data by time and stall number. For statistical analysis, we will conduct an analysis of variance. Our response variable will be number stepping and kicking during the first two minutes of the milking session and our main explanatory variable will be treatment. Additional covariates will be parity, DIM, group, presence of bimodal milking, and yield.

Results & Discussion

Our sample had 223 cows, of which 21% were in their first lactation. Preliminary results indicate that 27.4% of the cows had bimodal milk letdown. Descriptive data by treatment is presented in Table 1. Further analyses are currently being performed and will be available to be presented at the meeting.

Table 1. Descriptive data by treatment for 223 cows.

Treatment	n	Mean lactation number (SD)	Mean days in milk (SD)	Yield (SD) (kg)	Bimodal milk letdown (%)
Flow adjusted vacuum (44-49 kPa)	75	3.3[a] (1.6)	186.6[a] (115.8)	13.2[a] (2.8)	40.0
Constant high vacuum (49 kPa)	74	3.2[a] (1.8)	196.8[a] (111.7)	13.3[a] (3.0)	32.4
Constant normal vacuum (44 kPa)	74	3.3[a] (1.8)	184.9[a] (112.1)	13.3[a] (2.9)	9.5

[a,]Means without common superscript letter within the same column are significantly different (P<0.05).

Conclusion

No conclusion can be made at this time.

References

Browne, M., Silva Boloña, P., & Upton, J. (2024). Measurement of cow comfort during milking on different cluster removal settings through the use of leg-mounted accelerometers. *JDS Communications*, *5*(5), 462–467. https://doi.org/10.3168/jdsc.2023-0477

Edwards, J. P., Jago, J. G., & Lopez-Villalobos, N. (2014). Analysis of milking characteristics in New Zealand dairy cows. *Journal of Dairy Science*, *97*(1), 259–269. https://doi.org/10.3168/jds.2013-7051

Fernandes, S., Pereira, G., & Bexiga, R. (2023). Bimodal milk flow and overmilking in dairy cattle: risk factors and consequences. *Animal*, *17*(3). https://doi.org/10.1016/j.animal.2023.100716

Moore-Foster, R., Norby, B., Schewe, R. L., Thomson, R., Bartlett, P. C., & Erskine, R. J. (2019). Herd-level variables associated with delayed milk ejection in Michigan dairy herds. *Journal of Dairy Science*, *102*(1), 696–705. https://doi.org/10.3168/jds.2018-14561

Reinemann, D. J., van den Borne, B. H. P., Hogeveen, H., Wiedemann, M., & Paulrud, C. O. (2021). Effects of flow-controlled vacuum on milking performance and teat condition in a rotary milking parlor. *Journal of Dairy Science*, *104*(6), 6820–6831. https://doi.org/10.3168/jds.2020-19418

Wieland, M., Nydam, D. V., Heuwieser, W., Morrill, K. M., Ferlito, L., Watters, R. D., & Virkler, P. D. (2020). A randomized trial to study the effect of automatic cluster remover settings on milking performance, teat condition, and udder health. *Journal of Dairy Science*, *103*(4), 3668–3682. https://doi.org/10.3168/jds.2019-17342

Effects of switch level of automatic cluster removers on parlor efficiency, cow comfort, teat condition and udder health

Diego Racca[1], Pablo Leinecker[1] and Eial Izak[2*]

[1] Milkland S.A., El Fortin, Argentina

[2] Mastitis Prevention Services, Buenos Aires, Argentina

***Corresponding author's email: eizak@fibertel.com.ar**

Introduction

Automatic cluster removers (ACR), decreasing labor costs and improving parlor efficiency (Tarrant et al., 2012). Research has shown that increasing the ACR threshold from 0.2 to 0.8 kg/min, is effective to reduce milking duration (Edwards et al., 2013a; Upton et al., 2023). Wieland et al. (2020) found that increasing the ACR milk flow rate switch level, decreased the odds of short-term changes in teat condition. Stepping and kicking at the milking unit, is often coincident with overmilking (Cerqueira et al., 2017). The objective of this study was to determine the effects of switch level of ACR from 0.4 to 0.7 kg/min in a dairy herd with high prevalence of cows stepping and kicking at the milking unit on parlor efficiency, cow comfort, teat condition and udder health.

Material & methods

Data for this study were obtained from June to September 2024 at a commercial dairy farm located in the Province of Cordoba, Argentina. The average number of milking cows was 700, with 30 litres/cow/day and milked twice per day. A mid-line 22 unit DeLaval herringbone, swing-over milking system was used. Premilking preparation was performed by 3 milkers and consisted of forestripping, predipping, drying with individual paper towel and attaching the milking unit in a sequential milking routine. The ACR raised from 0.4 to 0.7 kg/min, in 0.1 kg/min weekly steps. Milking efficiency metrics evaluated were cows per hour, cows per operator per hour and litres of milk harvested per hour, according to the equations by Prendergast et al. (2023). Machine milking induced short-term changes to the teat condition, were visually assessed according to the scoring system described by Mein et al. (2001). Hind leg activity of cows stepping and kicking, was evaluated following the methodology of Meyer et al. (2021).

Overmilking time was recorded with VPR 200 (DeLaval), as the interval in seconds between beginning of overmilking and end of milking. The time period when milk flow ceased before the unit detached, lasted longer than 30 seconds was defined as overmilking. Monthly composite milk samples were analyzed for somatic cell count (SCC). SCC data were log-transformed (log_{10}) for subsequent analysis. Cow composite SCC data were used to categorise cows with subclinical mastitis (SM) or healthy (i.e. ≥ 200,000 vs. < 200,000 cells/mL, respectively). Clinical mastitis detection was performed by farm personnel during the premilking udder preparation and recorded with the farm management software (Dairy Comp 305). A general linear mixed model was used to analyze the effect of ACR switch level on milking efficiency, using the MIXED procedure in SAS 9.4. A mixed model procedure was used to analyze the effect of ACR switch level on short-term changes to the teat condition, using the Proc Glimmix command in SAS 9.4. A general linear mixed model was used to analyze the effect of ACR switch level on hind leg activity, using the GENMOD negative binomial regression procedure in SAS 9.4. To compare the effect of ACR switch level on monthly Linear SCS (LS) and prevalence of SM, a generalized linear mixed model was used with PROC MIXED in SAS 9.4. To compare the effect of ACR switch level on monthly incidence rate of clinical mastitis (IRCM), a generalized linear model with a logit link and a binomial distribution was fitted with PROC LOGISTIC in SAS 9.4.

Results & Discussion

The average milking efficiency for ACR 0.4 kg/min was 155 cows/h, 51 cows/operator/h and 2,333 L/h. The average milking efficiency for ACR 0.7 kg/min increased to 175 cows/h (+12.90%), 58 cows/operator/h (+13.73%) and 2,625 L/h (+12.52%). The average milking time decreased by 30 min per milking session for ACR 0.7 kg/min, in comparison with ACR 0.4 kg/min. Therefore, for an eight-hour work day, the total reduction was 45 days yearly or increase to 800 milking cows. A significant difference between treatments, was found for short-term changes in teat condition (teat congestion and ringing at the base of the teat) and hind leg activity (stepping and kicking). The prevalence for short-term changes in teat condition was 3.14% for cows in the ACR 0.7 kg/min, compared with 23.57% for ACR 0.4 kg/min. The prevalence for hind leg activity was 5.29% for cows in the ACR 0.7 kg/min, compared with 30.71% for ACR 0.4 kg/min. The effects of switch level of ACR on linear score and prevalence of subclinical mastitis were significant. The mean LS for ACR 0.7 kg/min and 0.4 kg/min was 4.3 vs. 5.5, respectively. The prevalence of SM for ACR 0.7 kg/min and 0.4 kg/min was 20.67% vs. 36.67%, respectively. A significant difference between treatments, was also found for monthly IRCM. The percentage was lower for cows in the ACR 0.7 kg/min (3.22%), compared with ACR 0.4 kg/min (7.91%).

The study found that increasing the cluster remover take-off milk flow threshold of the ACR from 0.4 to 0.7 kg/min, resulted in a significant decrease in milking duration and improved teat condition and cow comfort. A reduction in milking duration with increased ACR settings, has been shown in several studies (Edwards et al., 2013a; Wieland et al., 2020; Upton et al., 2023). According to Wieland et al. (2020), increasing the ACR settings, decreased the short-term changes to the teat condition. Teat congestion and

ringing at the base of the teat are related to impairment of the teats defense mechanisms, susceptibility to new intramammary infections (Zecconi et al., 1996) and animal welfare (Hillerton et al., 2002). Our findings demonstrated that increasing the cluster remover take-off milk flow threshold of the ACR from 0.4 to 0.7 kg/min, decreased LS, SM and IRCM. This study agreed with Cerqueira et al. (2017), establishing that overmilking led to significantly more stepping and kicking behavior during milking. Using properly adjusted and maintained ACR, prevents overmilking and enhance parlor throughput, teat condition, udder health and cow comfort.

Conclusion

Results of this study demonstrate that increasing the milk flow rate switch-point from 0.4 kg/min to 0.7 kg/min, reduced daily milking duration by 11.11%. An increased cluster removal milk flow threshold, reduced overmilking and lowering the risk of teat damage. Therefore, LS, SM and IRCM were reduced. Increased trends on milk production and herd size in Argentina, will further increase labour demand. An effective management and automation of ACR use, improve significantly milking and operator efficiencies. Adapting ACR switch-point settings offer a valuable opportunity to increase parlor efficiency, cow comfort and improve teat condition and udder health.

Association Between Milking System and *Streptococcus agalactiae* in Dairy Herds

Michael Farre[1*], Malene Vesterager Byskov[1], Marie Laurup Petersen[1], Lærke Boye Astrup[1]

Seges Innovation, Århus, Denmark [1]

Corresponding author's email: mifa@seges.dk

Introduction:

Streptococcus agalactiae (S. agalactiae) is a contagious mastitis pathogen that continues to pose a significant challenge in dairy herd management. It is primarily transmitted during milking through personnel or contaminated equipment, leading to persistent intramammary infections, elevated bulk tank somatic cell counts (BMSCC), reduced milk quality, and economic losses. Unlike environmental pathogens, S. agalactiae is specifically adapted to the udder environment, allowing subclinical infections to spread silently within herds.

Although automated milking systems (AMS) are designed to improve udder hygiene and reduce the risk of cross-contamination by eliminating manual milking and standardizing teat cleaning procedures, *S. agalactiae* remains a concern (Skarbye et al., 2021). Thus, other researchers have found that transmission in AMS herds were higher than in conventional milking systems (Deng et al., 2021). The pathogen's ability to persist and spread via milk residues or inadequately sanitized components means that even in automated systems, infections can spread. Differences in robot design, cleaning efficiency, and teat preparation protocols between manufacturers may influence the risk of within-herd transmission.

This study investigates one key aspect of S. agalactiae control in herds using robotic milking systems: potential differences in the prevalence of S. agalactiae-positive herds in relation to the brand of robotic milking system used in the dairy herd with conventional milking systems as gold standard.

Material & methods

Data for this study were collected from October 1, 2024, to October 1, 2025, in Denmark. Only dairy herds shipping milk and enrolled in the Dairy Herd Improvement (DHI) were considered, as information on milking robot manufacturers is only available for these herds. Approximately 91% of all dairy herds in Denmark are part of the DHI.
A herd was classified as infected with S. agalactiae if it held an official positive infection status at the latest national yearly surveillance. This definition implies that some herds may appear as infected despite not currently having S. agalactiae present, for example if the herd has never been officially re-tested to regain a negative status or if infected cows have been removed from the herd. Although infection could also be assessed based on sample results, the official infection status was used for this analysis to allow for rapid assessment.

To be included in the dataset, herds had to meet the following criteria throughout the entire period:

1. No change in *S. agalactiae* infection status.
2. No change in milking system.

Herds with both conventional milking and milking robots were classified as robot-milking herds if the same robot brand was maintained or not removed during the period.For each herd, a geometric mean of somatic cell count (SCC) and bacterial count from bulk tank milk samples taken during the study period were calculated. The number of annual cows per herd was also determined.
The number of dry-off treatments with antimicrobials per herd was derived from the national cattle database, because there is an association between prevalence of DCT and *S. agalactiae* herds. A cow could have up to two dry-off treatments during the study period if one occurred at the beginning and one at the end of the year.

Results & Discussion

In total, 1,692 milk-delivering herds met the inclusion criteria. Of these, 610 used one of the four milking robot brands on the Danish marked. The proportion of herds with an official positive *S. agalactiae* status varied slightly between milking systems (Table 1). The proportion of infected herds was 12.6% in the robot-milking group and 11.6% in the group with conventionally milked systems, indicating no substantial difference in infection prevalence between the two groups.
When comparing robot brands, infection rates ranged from 11.5% to 21.4%. However, the number of herds using Brand 3 and Brand 4 was small (14 and 13 herds, respectively). Therefore, observed differences should be interpreted with caution.
For herds with a positive *S. agalactiae* status, the average herd size, dry-off treatment rate, somatic cell count (SCC), and bacterial count were assessed. Conventional herds had on average larger herd sizes (425 cows) compared with robot-milking herds (231 cows).

Table 1. *S. agalactiae* status between AMS and conventional milking.

Brand	Status free	Status positive	Proportion positive
Brand 1.	365	53	12,7 %

Brand 2.	146	19	11,5 %
Brand 3.	11	3	21,4 %
Brand 4.	11	2	15,4 %
Conventional milking	949	125	11,6 %

The SCC and bacterial count were slightly higher in herds using Brand 3 and 4 robots, while dry off treatment with antimicrobials frequency varied between brands, being lowest in Brand 3 (0.05 per cow per year) and highest in Brand 4 (0.56 per cow per year) as illustrated in Table 2. Accordingly, the low frequency of dry off treatment with antimicrobials might partly explain the high number of *S. agalactiae*-positive Brand 3 herds. Contrary to this, the opposite correlation is observed in Brand 4 herds. Thus, the correlation between *S. agalactiae*-status and milking system should be interpreted with caution as several other variables affect the *S. agalactiae* status in addition to the milking system. Moreover, due to the limited number of infected herds for some of the brands, these differences are not considered robust.

Table 2. Descriptive statistics between AMS and conventional milking.

Brand	**Herd size**	**DCT**	**BMSCC**	**Bacteria count (CFU)**
Lely	296 (±156)	0,43 (±0,28)	170208 (±46195)	11343 (±5931)
DeLaval	334 (±303)	0,32 (±0,23)	181640 (±46811)	10131 (±6313)
GEA	231 (±40)	0,05 (±0,09)	196675 (±50819)	16984 (±9260)
BouMatic	247 (±107)	0,56 (±0,26)	218208 (±106416)	21206 (±4662)
Conventional milking	425 (±416)	0,41 (±0,40)	176150 (±53678)	9962 (±6209)

Overall, the results indicate that 1) the prevalence of *S. agalactiae* infection is similar between conventional and AMS herds, 2) suggesting that the choice of milking system or robot brand alone is unlikely to be a major risk factor. Nevertheless, the data also highlights substantial variation between individual milking systems, and further studies including a larger number of herds per robot brand are needed to confirm potential differences in infection risk and udder health indicators.

Conclusion

The findings indicate that the prevalence of *S. agalactiae* infection does not differ significantly between herds using automatic milking systems and those with conventional milking systems. This suggests that the choice of milking system or robot brand alone is not a key determinant of infection risk. However, variations in infection rates among individual herds highlight the importance of management practices, hygiene protocols, and regular monitoring in maintaining udder health. Future studies including larger datasets across robot brands are therefore recommended to further clarify potential design- or management-related differences influencing bacterial transmission.

References

Deng, Z., Koop, G., Hogeveen, H., Fischer, E. A. J., van den Borne, B. H. P., van der Tol, R., & Lam, T. J. G. M. (2021). Transmission dynamics of Staphylococcus aureus and Streptococcus agalactiae in a Dutch dairy herd using an automatic milking system. *Preventive Veterinary Medicine*, *192*, 105384. https://doi.org/10.1016/j.prevetmed.2021.105384

Skarbye, A. P., Krogh, M. A., Denwood, M., Bjerring, M., & Østergaard, S. (2021). Effect of enhanced hygiene on transmission of Staphylococcus aureus, Streptococcus agalactiae, and Streptococcus dysgalactiae in dairy herds with automatic milking systems. *Journal of Dairy Science*, *104*(6), 7195–7209. https://doi.org/10.3168/jds.2020-19635

Preliminary study: From cubicles to Bulk tank milk - testing protocol for total bacteria count identification

Frederik Damsgaard[1*], Michael Farre[2], Karl Pedersen[1], and Lærke B. Astrup[2].

[1] Aarhus University, Foulum, Denmark

[2] SEGES Innovation, Aarhus, Denmark

***Corresponding author's email: Fredam@anivet.au.dk**

Introduction

Total Bacteria Count (TBC) in the dairy industry has historically been used as a marker of both milk quality and hygiene (Hayes et al., 2001; Jayarao et al., 2004; Piepers et al., 2014; Ryšánek et al., 2009). TBC reflects the number of bacteria in a specific volume, often referred as colony-forming units pr. milliliter (CFU/mL) or individual bacteria count (IBC). Elevated TBC levels are associated with reduced milk quality and result in financial penalties for producers (Markusson, 2021; Piepers et al., 2014; Rodrigues et al., 2017; Twomey et al., 2025). At present, European legislation specifies only that raw milk must contain fewer than 100,000 CFU/mL (Markusson, 2021, Regulation (EC) no. 853/2004 of the European Parliament and of the Council, 2004). In Denmark, the dairy industry e.g. has reward-based payment scheme, which awards low TBC (Støve, 2017).

Specific species of bacteria sheds in different amounts, *Streptococcus uberis, Enterococcus faecalis, Enterococcus faecium, Streptococcus dysgalagtiae* and *Staphylococcus aureus* can be shed in substantial amounts (Rodrigues et al., 2017; Ryšánek et al., 2009; Svennesen et al., 2016). The bacterial diversity correlates negatively with elevated TBC, indicating that high TBC are often dominated by fewer bacteria (Rodrigues et al., 2017). However, the TBC and somatic cell count (SCC) positively correlates indicating higher SCC (>200,000 CFU/mL) correlates with high TBC (Rodrigues et al., 2017).

The current literature mainly addresses TBC in milk at a national or regional scale, while herd-specific studies often rely on sparse or infrequent data points. Thus, emphasizing that this investigation will be the first to be conducted on a large scale, including the whole herd. This study aims to investigate the origin and composition of bacteria contributing to elevated bulk tank milk (BTM) TBC at the herd level. To ensure that

elevated TBC values are not caused by equipment-related factors, herds are only enrolled after verification that milking, washing, and cooling systems are functioning correctly. The aim of this study is to provide new evidence on the sources of high TBC in herds and support evidence-based milk quality management strategies.

Material & methods

Study:

Herds must meet multiple inclusion criteria to ensure that data is repeatable. Each herd must have been subjected to a comprehensive inspection of milking, washing and cooling equipment to rule out technical issues as cause of elevated bulk tank bacterial count. All lactating cows from the eligible herd will be sampled three times with two-days intervals, the cows with high TBC will be included for further sampling. The sampling will be same interval as the screening. Alongside, the quarter milk samples from the high TBC cows which were identified during the screening process, teat swabs, where the teat skin on the same cows that are quarter milk sampled will also be swabbed, BTM sample and environmental samples such as bedding material will be collected. All samples will be analyses based on culture dependent methods the BTM and quarter milk samples will be analyses following the NMC guideline (NMC Laboratory Handbook on Bovine Mastitis, 2017) for handling BTM samples. The environmental and teat swabs are plated on the same selected agar as described in the NMC guideline (NMC Laboratory Handbook on Bovine Mastitis, 2017), in order to track the different bacteria throughout the dairy herd. All bacteria will be identified using MALDI-TOF ms for accurate identification.

Results & Discussion

This study represents to our knowledge the first comprehensive study to simultaneously screen all lactating cows and sample BTM, teat skin and the environment, to track the bacterial pathways within the herd. This investigation will identify cows shedding bacteria without any inflammation or sign of illness. By combining bacterial identification with origin tracking, it will be clarified whether elevated TBC arises primarily from cow-associated or environmental origins. The findings will provide valuable insight into developing improved management protocols for herds with high TBC unrelated to equipment issues and support early identification of potential bacterial-shedding cows within the herd.

Expected outcomes:

Results will be presented as bacteriological findings,
Correlation between TBC and SCC.
The proportion of cow contributing to the elevated TBC

Conclusion

The aim of this study is to identify cause of the elevated total bacterial count and how to address a dairy herd with such problems.

References

Hayes, M. C., Ralyea, R. D., Murphy, S. C., Carey, N. R., Scarlett, J. M., & Boor, K. J. (2001). Identification and characterization of elevated microbial counts in bulk tank raw

milk. Journal of Dairy Science, 84(1), 292–298. https://doi.org/10.3168/jds.S0022-0302(01)74479-7
Jayarao, B. M., Pillai, S. R., Sawant, A. A., Wolfgang, D. R., & Hegde, N. V. (2004). Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. Journal of Dairy Science, 87(10), 3561–3573. https://doi.org/10.3168/jds.S0022-0302(04)73493-1
Markusson, H. (2021). Total bacterial count as an attribute for raw milk quality. https://stud.epsilon.slu.se/17124/1/markusson_h_210809.pdf
NMC Laboratory Handbook on Bovine Mastitis (Third edition). (2017). National Mastitis Council.
Piepers, S., Zrimšek, P., Passchyn, P., & De Vliegher, S. (2014). Manageable risk factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds. Journal of Dairy Science, 97(6), 3409–3419. https://doi.org/10.3168/jds.2013-7203
Rodrigues, M. X., Lima, S. F., Canniatti-Brazaca, S. G., & Bicalho, R. C. (2017). The microbiome of bulk tank milk: Characterization and associations with somatic cell count and bacterial count. Journal of Dairy Science, 100(4), 2536–2552. https://doi.org/10.3168/jds.2016-11540
Ryšánek, D., Zouharová, M., & Babák, V. (2009). Podílejí se hlavní patogeny mléčné žlázy skotu na celkovém počtu mikroorganismů v syrovém mléce? Acta Veterinaria Brno, 78(3), 455–461. https://doi.org/10.2754/avb200978030455
Støve, J. (2017, June 26). Værd at vide om kim i mælken og hvad du gør ved forhøjet kimtal.
https://www.landbrugsinfo.dk/public/7/a/4/malkning_malkekvalitet_vard_at_vide_om_kim_i_malken_og_hvad_du_gor_ved_forhojet_kimtal
Svennesen, L. ;, Bennedsgaard, T. W. ;, Pedersen, K. ;, & Klaas, I. C. (n.d.). General rights Short time variation in daily shedding of Strep. agalactiae and Staph. aureus determined by bacterial culture and PCR test.
Twomey, L., Furey, A., O'Brien, B., Beresford, T., & Gleeson, D. (2025). Minimizing Bacterial Counts in Bulk Tank Milk: A Review with a Focus on Chlorine-Free Cleaning. In Dairy. https://doi.org/10.3390/dairy6010007

Overcoming Barriers to Milk Recording Adoption: A Synthesis of Technological, Farmer Engagement and Milking Infrastructure Challenges

L. Power[1], J. Upton[2]

[1] Lucy Power, University College Dublin, Ireland

[2] Dr. John Upton, Teagasc Moorepark, Ireland

Corresponding author's email: lucy.power@teagasc.ie

Introduction

Milk recording underpins herd profitability, productivity, herd-health management and informed decision making by providing individual cow data (Balaine et al., 2020). Achieving 90% participation in milk recording across dairy herds by 2030 has been identified as a strategic priority for the Irish dairy sector (Department of Agriculture, 2022). In Ireland, recording herds achieve higher yields (+177.5 L/cow), lower somatic cell counts and improved gross margins (+€39/cow) (Burrell et al., 2024). Despite these benefits, national participation rates remain lower than those of comparable international dairy industries (ICAR, 2025). This paper synthesises technological, behavioural and infrastructural barriers limiting adoption and identifies practical pathways to increase uptake.

Material & Methods

A structured literature review (2000–2025) was conducted to synthesise barriers to milk recording adoption and associated mitigation strategies. ScienceDirect and Google Scholar were searched using combinations of terms including "milk recording adoption", "milk meter technology", and "dairy technology uptake". Studies addressing milk recording technology, adoption behaviour or infrastructure constraints in dairy systems were included, while non-dairy and non-English publications were excluded. Identified barriers were categorised into technological, behavioural and infrastructural domains to enable thematic synthesis.

Results & Discussion

Barriers to Milk Recording Adoption

Technological constraints primarily relate to equipment reliability, sensor limitations, interoperability challenges and complex reporting systems that hinder effective data integration (Steeneveld et al., 2015, Burrell et al., 2024). Limited reconciliation between bulk tank and individual cow data, coupled with restricted access to timely technical support, further undermines farmer confidence in system performance (Balaine et al., 2020).

Behavioural and economic factors also influence adoption. Farmer engagement barriers encompass knowledge, beliefs, behaviour and economic factors. Perceived lack of immediate or long-term benefits, negative past experiences, mistrust of data and satisfaction with current herd performance reduce motivation to adopt. Engagement is further constrained by limited advisory support, low participation in discussion groups, reluctance to involve external personnel, intention–behaviour gaps, increased workload (associated labour cost), upfront costs and absence of direct financial incentives (Dillon et al., 2015).

Structural limitations within milking infrastructure present additional constraints. Barriers include outdated or incompatible parlour systems, absence of individual cow identification or meters, physical layout constraints and high upgrade or retrofitting costs. Adoption may also be influenced by farm location, perceived superiority of alternative systems (e.g., robotic milking) and potential disruption during installation, particularly for smaller herds (Burrell et al., 2024).

Potential Solutions

Technological solutions include standardised calibration, subsidised ICAR-approved meters, improved software integration, automated reconciliation of bulk tank and individual cow data, user-friendly reporting dashboards and timely technical support (ICAR, 2023). Building on this, a Farmer Back-to-Farmer (Fb2F) approach engages farmers, researchers and other stakeholders in participatory technology development, ensuring innovations are relevant, usable and widely accepted by end users. (Rhoades and Booth, 1982).

Overcoming behavioural constraints necessitates farmer-led, multi-actor engagement strategies grounded in Social-Cognitive Theory, emphasising peer learning, confidence building and progressive behaviour change (Crane, 2014)(Bandura, 1986). Practical strategies include targeted agricultural education, peer-to-peer discussion groups, participatory decision-making, clear communication of costs and benefits, financial supports and flexible service options (Burrell et al., 2024). Together, these measures help farmers understand the value of milk recording and achieve long-term adoption.

Infrastructure-focused solutions include standardised cow identification, compatibility standards across technologies, on-farm audits, advisory support during installation and phased or grant-supported retrofit to reduce costs and installation disruption, particularly for smaller herds or farms in challenging locations (Burrell et al., 2024).

Overall, increasing adoption requires a whole-system approach that simultaneously addresses technological trust, behavioural change and structural compatibility.

Conclusion

Milk recording delivers measurable improvements in yield, udder health and profitability, yet adoption remains limited by interacting technological, behavioural and infrastructural constraints. Increasing uptake requires coordinated action across these domains, combining reliable technology, behaviourally informed engagement strategies and compatible infrastructure design. Such an integrated approach is necessary to achieve sustained, high-participation milk recording systems.

References

Balaine, L., E. J. Dillon, D. Läpple, and J. Lynch. 2020. Can technology help achieve sustainable intensification? Evidence from milk recording on Irish dairy farms. Land use policy 92.

Bandura, A. 1986. The Explanatory and Predictive Scope of Self-Efficacy Theory. Journal of Social and Clinical Psychology 4(3):359-373.

Burrell, A. M., L. Balaine, S. Clifford, M. McGrath, D. A. Graham, F. McCoy, E. Dillon, and Á. Regan. 2024. A multi-methods, multi-actor exploration of the benefits and barriers to milk recording on Irish farms using the COM-B model. Prev Vet Med 227:106195.

Crane, T. A. 2014. Bringing Science and Technology Studies into Agricultural Anthropology: Technology Development as Cultural Encounter between Farmers and Researchers. Culture, Agriculture, Food and Environment 36(1):45-55.

Department of Agriculture, F. a. t. M. 2022. Food Vision 2030: Pathways to Sustainable Food Systems.

Dillon, E. J., T. Hennessy, and J. Cullinan. 2015. Measuring the economic impact of improved control of sub-clinical mastitis in Irish dairy herds. The Journal of Agricultural Science 153(4):666-675.

ICAR. 2023. Guidelines for Dairy Cattle Milk Recording.

ICAR. 2025. Global Standard for Livestock Data Online Database.

Rhoades, R. E. and R. H. Booth. 1982. Farmer-back-to-farmer: A model for generating acceptable agricultural technology. Agricultural Administration 11(2):127-137.

Steeneveld, W., J. C. M. Vernooij, and H. Hogeveen. 2015. Effect of sensor systems for cow management on milk production, somatic cell count, and reproduction. Journal of Dairy Science 98(6):3896-3905.

DISTRIBUTION: Swedish University of Agricultural Sciences
Department of Applied Animal Science and Welfare
Box 7024 750 07 Uppsala
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