Heifer Mastitis Conference 2007

June 24-26, 2007
Ghent, Belgium

Final Program & Abstract Book
Heifer Mastitis Conference 2007

June 24-26, 2007

Pre-Conference Symposium on Coagulase-Negative Staphylococci in Bovine Mastitis

June 24, 2007

Ghent, Belgium
Preface

Dear participant, contributor, colleague, friend …

It is with great pleasure and excitement that I’m writing these words of welcome. Currently people from as far as Japan, New-Zealand, Canada and Argentina have registered for what we have called: “The heifer mastitis conference”. What was just an idea two years ago has grown into a reality…!

Never before has a conference focused solely on heifer mastitis, a problem that has plagued farmers and their advisors for so many years. The scientific and organizing committees have compiled an excellent program full of prominent lecturers replete with high quality presentations and posters from researchers currently working on the subject. As you read this text we are at the start of two days packed with state-of-the-art information and opportunities to learn and discuss, to exchange views, to think about innovative approaches … enjoy!

We realize that the coagulase-negative staphylococci are the primary cause of mastitis in heifers and moreover the most common cause of subclinical mastitis in numerous countries. Thus we added a full day of sessions on this important topic: coagulase-negative staphylococcal mastitis. It is time to finally get some answers about the relevance of these bacteria (to treat or not to treat …), how to improve prevention and how to differentiate the species (if needed at all)!

We have received excellent support for this meeting. I want to thank you all for being here and participating in the discussions, the lectures, the reception, the medieval dinner … Also, the sponsors have been very generous making the organisation of this scientific conference so much easier. Thank you!

I sincerely hope you all have a great time and that all your scientific expectations are met. Do not forget to breathe the historical atmosphere that you will find abundantly present in our lovely conference city at the confluence of the rivers “Schelde” and “Leie”.

Welcome to Ghent!

On behalf of the organizing and scientific committees,

Sarne

Sarne De Vliegher, chair
Committees

Local Organising Committee (Belgium)

Dr. Sarne De Vliegher (Ghent University) (Conference Chair)
Prof. Dr. Aart de Kruif (Ghent University)
Prof. Dr. Geert Opsomer (Ghent University)
Prof. Dr. Johanne Detilleux (Université de Liège)

International Scientific Committee

Prof. Dr. Herman W. Barkema (University of Calgary, Canada)
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Prof. Dr. Johanne Detilleux (Université de Liège, Belgium)
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Prof. Dr. Larry Fox (Washington State University, United States)
Dr. Theo Lam (Dutch Animal Health Service, The Netherlands)
Dr. Peter Mansell (University of Melbourne, Australia)
Prof. Dr. Geert Opsomer (Ghent University, Belgium)
Prof. Dr. Ynte H. Schukken (Cornell University, United States)
ing. Otlis Sampimon (Dutch Animal Health Service, The Netherlands)
Dr. Ruth Zadoks (Cornell University, United States)
Prof. Dr. Alfonso Zecconi (University of Milan, Italy)

Acknowledgement

The Committees of the Heifer Mastitis Conference gratefully acknowledge the support of the following institutions and companies and wish to thank them for their contribution and support.

Institution
Research Foundation Flanders – (FWO)

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Vétoquinol

Bronze Partners
Alltech
Bayer Health Care Animal Health
CID-Lines
GD

Others
Campina
Corilus
General Information

VENUE AND DATES

The Heifer Mastitis Conference takes place at the prestigious “Aula” of Ghent University, 24-26 June 2007.

Aula University Ghent, Volderstraat 9, 9000 Ghent

LANGUAGE

The official language of the Conference is English.

REGISTRATION DESK

OPENING HOURS:
Sunday, June 24 08:30-19:00
Monday, June 25 07:30-18:00
Tuesday, June 26 08:30-18:00

COFFEE BREAKS AND MEALS

Coffee/tea and lunches are included in the registration fee and will be served daily in the Peristilium.

CLIMATE AND DRESS

The weather in Ghent at this time of the year is usually sunny with temperatures around 18-20°C. An umbrella might be useful as showers can occur. Dress will be informal throughout the Conference.

TIME ZONE

The time zone in Ghent is GMT + 1 hour.

BANKS AND POST OFFICE

Most banks opens at 09h00 and close around 16h00, from Monday through Friday. They are generally closed for lunch between 12h30-14h00. Post offices are generally open between 09h00 and 16h00. Please, note that there will be no exchange facilities at the meeting venue.

ELECTRICITY

The voltage in Belgium is 220V, 50 Hz.
Social Program

WELCOME RECEPTION

Sunday, 24 June (18h30-20h00)
(Aula, Volderstraat, 9000 Ghent)

During this drink, everybody will have the opportunity to meet old friends and make new ones.

CONFERENCE DINNER (walking dinner)

Monday, 25 June (20h00-23h30)
(Castle of the Counts, Veerleplein, Ghent)

The GRAVENSTEEN is the Dutch name for the 'castle of the count'. The counts of Flanders had castles built in the principal cities of the county. Because they had to maintain law and order, they continuously had to move from one city to the other. Therefore, they disposed of a castle in most cities where they wanted to stay for a few months. The castle of Ghent is the only one, which survived the centuries more or less intact.

Archaeological excavations have proved that three fortified castles constructed in wood must have stood on the site of today's Gravensteen. Already around the year 1000 the first stone castle must have been erected here. Parts of this, such as the chimney and the fireplace, can still be found in the walls of the lower floors of the main tower.

Fillips of Alsasse who was count of Flanders between 1157 and 1191 have constructed the Gravensteen, like we know it today. He took part in one of the crusades and died during the siege of Akko in the Holy Land. The opening in the form of a cross, right above the main entrance gate, proves that he already had taken part in a crusade when the Castle was built around 1177-1178. The Gravensteen functioned as the center of the Count's power during the early Middle Ages. This is somewhat symbolized by the main keep or 'donjon' (tower) from where one can have a panoramic view over the city. Next to the castle lies the Veerleplein (Veerle square), the place where public executions took place. The Gravensteen has been used in later times for different purposes. After the counts moved to more comfortable mansions in the later centuries, it was used as the Mint and later as the main prison of Ghent. In the nineteenth century a cotton plant was installed here. In the inner court little houses where built for the textile workers of the plant.

Today, the Gravensteen has been beautifully restored. It is still partially surrounded by the medieval moat. It can be visited all through the year.
ABSTRACT BOOK
All accepted abstracts are published in this Program & Abstract Book, included in your Conference Bag.

Manuscripts will be published in a special issue of the Journal “Veterinary Microbiology”.

CERTIFICATE OF ATTENDANCE
A certificate of attendance is included in your registration documentation.

POSTER EXHIBITION
The poster exhibition can be viewed during coffee breaks and lunch hours.

INSTRUCTIONS FOR ORAL PRESENTERS
• Please, hand in your presentation on CD-ROM (or USB-stick) at the room technician at least two hours before your presentation is scheduled.
• Speakers are requested to come to the meeting room at least 5 minutes prior to the start of the session and identify them to the session moderator.

INSTRUCTIONS FOR SESSION MODERATORS
• Before the session, check with the room technician if there are no-shows or last-minute changes in the program.
• You must be present in the room 5 minutes before the start of your session to confirm the names of the presenting authors.
• Your task is to moderate the session to facilitate both the presentations and the ensuing discussion.
• You should introduce each invited and key-note speaker.
Program Pre-Conference Symposium on Coagulase-Negative Staphylococci in Bovine Mastitis
Sunday 24 June 2007

09h45 Welcome and general introduction
S. De Vliegher, Belgium

10h00-11h15 SESSION 1: SIGNIFICANCE

Moderator: L.K. Fox, United States

Invited lectures:
10h00 001 CNS: emerging pathogens
S. Pyörälä, Finland

10h25 002 CNS mastitis: nothing to worry about
Y.H. Schukken, United States

10h50 Discussion

11h15 Coffee break - poster viewing

11h45-13h00 SESSION 2: IDENTIFICATION AND DIFFERENTIATION

Moderator: T.J. Lam, The Netherlands

Invited lectures:
11h45 003 The quest for the perfect test: phenotypic versus genotypic identification of CNS
P.L. Ruegg, United States

12h10 004 Species identification of CNS: genotyping as the gold standard
R.N. Zadoks, United States

12h35 Discussion

13h00 Lunch

14h00-15h15 SESSION 3: ANTIBIOTIC RESISTANCE/ VIRULENCE AND PERSISTENCE

Moderator: S.P. Oliver, United States

Invited lectures:
14h00 005 Antibiotic resistance in CNS: an emerging problem?
D. Mevius, The Netherlands

14h25 006 CNS: not so different from Staphylococcus aureus?
S. Taponen, Finland

14h50 Discussion
15h15   Coffee break - poster viewing

15h45-17h15 SESSION 4: PRACTICAL APPROACH/ HUMAN PERSPECTIVE

Moderator: P. Mansell, Australia

Invited lecture:
15h45 007 Prevalence of CNS on dairy farms in The Netherlands
       O. Sampimon, The Netherlands

Keynote lecture:
16h10 008 Role of CNS in human disease
       G. Verschraegen, Belgium

16h55 Discussion

17h15-17h45 Concluding discussion with all speakers

Moderator: L.K. Fox, United States

18h30 Welcome Reception at the Aula of the Ghent University
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Heifer Mastitis Conference

II

Program Heifer Mastitis Conference
**Monday 25 June 2007: day 1**

08h45 **Welcome and general introduction**  
S. De Vliegher, Belgium

09h00-10h15 **SESSION 1: EPIDEMIOLOGY - PREVALENCE AND INCIDENCE OF (SUB) CLINICAL HEIFER MASTITIS**

**Moderator:** T.G. Lam, The Netherlands

**Invited lecture:**  
09h00 009 **Prevalence and incidence of clinical and subclinical heifer mastitis**  
*L.K. Fox, United States*

**Accepted lectures:**  
09h35 010 **Prevalence and incidence of (sub)clinical mastitis in heifers in a random sample of dairy herds in the Netherlands**  
*B.H.P. van den Borne, G. van Schaik, M. Nielen and T.J.G.M. Lam*

09h50 011 **Incidence of mastitis and bacterial findings at acute clinical mastitis in Swedish primiparous cows - influence of breed and stage of lactation**  
*K. Persson Waller, B. Bengtsson, A. Lindberg, H. Unnerstad and A. Nymann*

10h05 **Discussion**

10h15 **Coffee break – poster viewing**

10h45-12h00 **SESSION 2: ASSOCIATED PATHOGENS AND SOURCES OF HEIFER MASTITIS**

**Moderator:** P.R. Ruegg, United States

**Invited lecture:**  
10h45 012 **Types and sources of mastitis pathogens associated with heifer mastitis**  
*S.P. Oliver, United States*

**Accepted lectures:**  
11h20 013 **Experimental model of bovine clinical mastitis caused by Staphylococcus chromogenes**  
*H. Simojoki, T. Orro, S. Taponen and S. Pyörälä*

11h35 014 **Frequency of teat canal closure in prepartum heifers and its relation to udder health**  
*V. Krömker and J. Friedrich*

11h50 **Discussion**

12h00 **Lunch**
SESSION 3: PATHOPHYSIOLOGY AND IMMUNITY RELATED TO HEIFER MASTITIS

Moderator: A. Zecconi, Italy

Keynote lecture:
13h45 015 Cumulative physiological events modulate the inflammatory response of the bovine udder to Escherichia coli infections around parturition
Ch. Burvenich, Belgium

Accepted lectures:
14h35 016 In vitro growth of Staphylococcus aureus in primiparous and multiparous blood and milk neutrophils during early lactation
J. Mehrzad and C. Burvenich

14h50 017 Heifer and quarter level factors associated with periparturient blood and milk neutrophil viability and concentration
S. Piepers, S. De Vliegher, E. Meyer, K. Demeyere, A. de Kruif and G. Opsomer

15h05 Discussion

15h15 Coffee break – poster viewing

SESSION 4: IMPACT OF HEIFER MASTITIS: ASSOCIATION WITH SCC, PRODUCTION, CULLING, AND FERTILITY

Moderator: S. Pyörälä, Finland

Invited lecture:
16h00 018 Impact of heifer mastitis: association with somatic cell count, production, culling and fertility
H.W. Barkema, Canada

Accepted lectures:
16h35 019 Influence of intramammary infections after calving on the risk of subclinical mastitis in the first 100 days of lactation
R. Piccinini, V. Daprà and A. Zecconi

16h50 020 Prevalence and effects of mastitis pathogens isolated from dairy heifers in Argentina on milk production and lactation somatic cell count
E. Izak, J. C. Bonazza, L. C. Perren and J. P. Araya

17h05 021 Heifer mastitis: it takes money
K. Huijps, S. De Vliegher, and H. Hogeveen

17h20 Discussion

17h30-18h00 General discussion with all speakers of the day
Moderator: P. Mansell, Australia

20h00 Conference dinner in medieval castle “Gravensteen” in city centre of Ghent
Tuesday 26 June 2007: day 2

08h55 Welcome
S. De Vliegher, Belgium

09h00-10h55 SESSION 5: CONTROL OF HEIFER MASTITIS I - ANTIMICROBIAL TREATMENT

Moderator: R.N. Zadoks, United States

Invited lecture:
09h00 022 Control of Heifer Mastitis I - Antimicrobial Treatment
S.C. Nickerson, United States

Accepted lectures:
09h35 023 Evaluation of the California Mastitis Test as a precalving treatment selection tool for Holstein heifers

09h50 024 Periparturient use of micronised Procaine Penicillin to reduce the risk of mastitis in heifers
M. Bryan and K. Taylor

10h05 025 Effect of periparturient treatment with penethamate hydriodide on the udder health of heifers in S. aureus problem herds
P. Winter, M. Kreiger, J. Hofer and G.M. Friton

10h20 026 Factors associated with the risk of antibiotic residues and intramammary pathogen presence in milk from heifers administered prepartum intramammary antibiotic therapy

10h35 Discussion

10h55 Coffee break – poster viewing

11h30-12h30 SESSION 6: CONTROL OF (HEIFER) MASTITIS II – GENETICS

Moderator: S.P. Oliver, United States

Invited lecture:
11h30 027 Genetic factors affecting susceptibility to udder pathogens
J.C. Detilleux, Belgium

Accepted lecture:
12h05 028 Genetic parameters for clinical mastitis in primi- versus multiparous cows
S. Bloemhof, G. de Jong and Y. de Haas

12h20 Discussion

12h30 Lunch
14h00-15h10 SESSION 7: CONTROL OF (HEIFER) MASTITIS III - NUTRITION

Moderator: G. Opsomer, Belgium

Keynote lecture:
14h00 029 Control of heifer mastitis by nutrition
J. Heinrichs, United States

Accepted lecture:
14h50 030 Feeding in the period around parturition and associations with subclinical and clinical mastitis of primiparous cows in early lactation
A.-K. Nyman, U. Emanuelson, and K. Persson Waller

15h05 Discussion
15h15 Coffee break

15h45-17h35 SESSION 8: CONTROL OF HEIFER MASTITIS IV - MANAGEMENT

Moderator: H.W. Barkema, Canada

Invited lecture:
15h45 031 Management approaches to controlling heifer mastitis
S. McDougall, New Zealand

Accepted lectures:
16h20 032 Heifers teat sprayed in the dry period have reduced Streptococcus uberis teat-end contamination and less Streptococcus uberis intramammary infections at calving

16h35 033 Efficacy of vaccination against staphylococci in heifers: a review and new data
J.R. Middleton

16h50 034 Individual and herd factors associated with mastitis and bacterial isolates from quarter milk samples drawn from fresh heifers
O. Østerås, A. Cathrine Whist, L. Solverød

17h05 035 Relationships between milk flow traits and udder health in Holstein heifers during the first 120 days of lactation
V. Daprà, M. Tonni, R. Piccinini, A. Zecconi, L. Bava, A. Sandrucci, A. Tamburini, M. Zucali

17h20 Discussion

17h40-18h00 General discussion with all speakers of the day

Moderator: A. de Kruif, Belgium

18h00h Closing of the conference
**Poster Presentations**

P013 Aetiology and risk factors for new intramammary infection in dairy heifers around calving  
N. Bareille, B. Djabri, F. Beaudeau, A. Robert and H. Seegers

P014 Characterisation of the bovine innate immune response following deliberate intramammary infection with *Streptococcus dysgalactiae*  

P015 Kinetics of serum antibodies against *S. aureus* and *E. coli* in Heifers vaccinated against bovine mastitis and correlation with the level of these antibodies in milk  
S. Casademunt, A. Foix, P. Lorenzo, A. Prenafeta and J. Tórtora

P016 Low pathogen load versus high non pathogen infusion in deliberate bovine intramammary challenges  
M. Daly, C. Beecher, K. Klostermann, C. Hill, R.P. Ross, W. Meaney and L. Giblin

P017 Effect of milking frequency on udder health of dairy heifers  
D. Gleeson, B. O'Brien and D. Berry

P018 Metabolic Changes in lactating cows during experimentally induced mastitis with endotoxins or *Escherichia coli*  
A. Gubbiotti, G. Bertoni, E. Trevisi and C. Burvenich

P019 Growth hormone, insulin-like growth factor and prolactin during clinical *Escherichia coli* mastitis in the bovine  
A. Gubbiotti, V. Van Merris, H. Dosogne, R.M. Bruckmaier, J. Blum, G. Bertoni, E. Trevisi and C. Burvenich

P020 Effect of prepartum intramammary infusion of Albadry Plus on the rate of postpartum *Staphylococcus aureus* infections of the heifer’s mammary glands  
H. Hamali

P021 Auction stress - predisposing factor of mastitis in heifers ?  
J. Hamann, D. Kleinschmidt, F. Reinecke and R. Redetzky

P022 Effect of organic zinc supplementation on somatic cell count in cow milk  
J. Illek, K. Batová and D. Kumprechtová

P023 Influence of lactation somatic cell count on milk production of dairy heifers in Argentina  
E. Izak, J. C. Bonazza, L. C. Perren and J. P. Araya

P024 Mastitis incidence: The influence of farmers' behaviour and attitudes  
J. Jansen, R.J. Renes, G. van Schaik, B. van den Borne and T. Lam

P025 Bovine mononuclear leukocyte subpopulations in colostrum and peripheral blood in response to mastitis during the periparturient period  
P026 Polymorphonuclear leukocytes express CD14 and CD44 during inflammation induced by muramyl dipeptide and lipopolysaccharide
T. Langrova, Z. Sladek and D. Rysanek

P027 Variation in gene expression of the TLR4 pathway in unstimulated circulating bovine neutrophils during early and mid-to-late lactation
S. Lefever, G. Vermeulen, A. De Ketelaere, L. Senepart, B. De Spiegeleer, L. Peelman, and C. Burvenich

P028 The prevalence of Heifer Mastitis due to Leptospirosis
T.G. Massoud

P029 A Study of the Incidence of Bacterial Species Associated With Intramammary infections in Dairy Heifers in Mashhad, Iran
M. Mohsenzadeh, A. Fallah-Rad and M. Amirani

P030 Pro- and anti-inflammatory effects of in vitro Escherichia coli phagocytosis by bovine neutrophils
S. Notebaert and E. Meyer

P031 Associations between management practices in the period around parturition and somatic cell counts of primiparous cows in early lactation
A.-K. Nyman, U. Emanuelson and K. Persson Waller

P032 Molecular analysis of 'no growth' milk cultures from mastitic cows
F. O’Halloran, J. Flynn, W.J. Meaney and R.P. Ross

P033 Incidence of intramammary infection at parturition for first calf heifers and multiparous cows
M.J. Paape, D.D. Bannerman and M.E. Bowman

P034 A critical evaluation of the control of clinical and subclinical mastitis in heifers in pasture-based Australian dairy herds
J Penry and P Mansell*

P035 Energy balance of primiparous and multiparous Holstein cows following short dry periods

P036 Effect of short dry periods on milk production, composition and reproductive parameters in Holstein cows

P037 IL-8 concentrations in milk of non-infected and naturally infected Holstein heifers with subclinical CNS mastitis postpartum
R. Pichler, L. Podstatzky-Lichtenstein, A. Tichy and P. Winter

P038 Peptibolomics of the cows’ udder: peptide profiling of the teat canal
L. Senepart, V. Vergote, A. Pezeshki, S. Bodé, S. Lefever, B. Baert, K. Peremans, C. Burvenich and B. De Spiegeleer
P039 Thyroid hormones and cortisol in lactating cows after intramammary LPS administration or *Escherichia coli* administration

P040 Effects of myramyldipeptide and lipopolysaccharide on CD44 expression on macrophages during the resolution of bovine mammary gland inflammatory response
Z. Sládek and D. Ryšánek

P041 Staphylococcus aureus mastitis in first parity dairy cows in Hungary
A. Tirián, M. Kovács, E. Brxdl, O. Szenci, L. Könyves, V. Jurkovich and E. Ungvári

P042 Subclinical Mastitis Accompanied Ketosis in Cows in Al-Diwaniya Province / Iraq
J. A.A. Al-Sa'a'idi
Abstracts Pre-Conference Symposium on Coagulase-Negative Staphylococci in Bovine Mastitis
SESSION 1: SIGNIFICANCE

001  CNS – emerging pathogens

Satu Pyörälä*, Suvi Taponen
University of Helsinki, Faculty of Veterinary Medicine,
Department of Production Animal Sciences, Saarentaus, Finland
*Corresponding author: satu.pyorala@helsinki.fi

Epidemiology and significance CNS infections: Coagulase-negative staphylococci (CNS) have traditionally been considered as minor pathogens, especially in comparison with major pathogens. Their importance has increased, as CNS have become the predominant pathogens isolated from subclinical mastitis in several countries\textsuperscript{1,2}. In Finland, CNS were isolated in a nationwide survey from 50% of the quarters positive for bacterial growth\textsuperscript{1}. In a similar survey in Norway, the respective prevalence of CNS was 16%\textsuperscript{3}, but the detection limit for positive diagnosis was higher. The proportion of CNS among bacteria causing clinical mastitis is still in many countries very low\textsuperscript{4}. In the practice area of the Faculty of Veterinary Medicine, University of Helsinki, Finland, more than 20% of bacterial isolates from milk samples from clinical mastitis were CNS\textsuperscript{5}. A number of CNS species, identified with methods based on phenotype, have been isolated from bovine mastitis. The two species reported most often are \textit{S. chromogenes} and \textit{S. simulans}, but also \textit{S. hyicus} and \textit{S. epidermidis} have frequently been isolated\textsuperscript{6,7,8,9}.

The prevalence of CNS mastitis is higher in primiparous cows than in older cows\textsuperscript{2,10,11}. In intensive management systems, the prevalence of quarters of precalving heifers infected with CNS may exceed 50%\textsuperscript{12,13}, whereas in pasture-based grazing systems a lower prevalence (16%) has been reported\textsuperscript{14}. CNS have been isolated from the mammary gland and teat apices of heifers as young as 10 months old\textsuperscript{15}. CNS species causing infections in heifers and cows of different age may partially differ. \textit{S. chromogenes} has been found to be the major CNS species in heifers and primiparous cows\textsuperscript{9,12,16}, whereas \textit{S. simulans} has been isolated especially from cows in later lactations\textsuperscript{6,8}. Multiparous cows in general become infected with CNS in later lactation, whereas primiparous cows usually already have the infection in the beginning of lactation\textsuperscript{18}.

CNS infections usually are subclinical or show mild clinical signs\textsuperscript{6,8,9}. In quarters infected with CNS, the increase in the milk SCC is less than caused by major pathogens. The direct economical impact of high SCC depends on the violation limits for poor quality milk or possible quality premiums paid for high quality milk, which considerably differ between countries. Studies on CNS intramammary infections in relation to milk production have shown a slightly decreased milk production\textsuperscript{17,18}.

Management of CNS mastitis: Traditionally CNS have been considered as normal skin flora which as opportunistic bacteria can cause mastitis\textsuperscript{13}. Control measures against contagious mastitis pathogens such as post-milking teat disinfection reduce CNS infections in the herd. Discontinuation of teat dipping significantly increased prevalence of infections with \textit{Corynebacterium bovis} and CNS\textsuperscript{19}. Some CNS isolated from mastitis may be opportunists from the environment, but it is very likely that at least the main species infecting bovine mammary gland are specialized for udder environment. Heifers are much more likely to be infected with CNS. In solving CNS mastitis problems, focus should therefore be on the heifers, i.e. their environment, feeding and management before calving. Welfare and comfort of heifers may be significant factors for good udder health. Prepartum intramammary antibiotic therapy for heifers has been suggested to reduce CNS mastitis during first lactation\textsuperscript{13}, but in a recent study in several herds from U.S. and Canada\textsuperscript{20}, no clear advantage from this practice
could be shown. In a meta-analysis on the efficacy of dry cow antimicrobial treatment\(^\text{21}\), no significant benefit from dry cow therapy was found for the prevention of CNS infections.

Mastitis caused by CNS is not a therapeutic problem, as it generally responds well to antimicrobial treatment (Taponen et al. 2006). Intramammary treatment with antimicrobials can be recommended for quarters with persisting CNS mastitis during lactation. A single isolation of CNS from a quarter does not economically justify antimicrobial treatment, if no clinical signs are present. For treatment of persistent CNS intramammary infections, treatment at drying-off remains a good tool.

References:

CNS mastitis: nothing to worry about

Ynte H. Schukken and QMPS staff
Quality Milk Production Services, Cornell University, Ithaca, USA.

Introduction: Coagulase Negative Staphylococci (CNS) intramammary infections have been associated with an increase in somatic cell counts of affected cows (2,3). However, the importance of this intramammary infection is debated. Classically, CNS infections were classified as minor pathogens and their importance as an independent cause of subclinical or clinical mastitis was judged to be limited (2). However, more recent studies propose that infections with CNS may cause more serious harm (3). CNS infections have been studied in pre-partum treatment trials in heifers and a bacteriological cure was associated with a decrease in somatic cell counts (SCC) (1). Most studies lack a sufficient sample size to evaluate both the mean effect of CNS and the variability of the effect. A large variability would imply that in some cows and herds, CNS infections might play a major role in udder health and milk quality. In this presentation we will present a very large data set on intramammary infections and the associated SCC in dairy cows. The objective is to study the impact of CNS infections on cow SCC and on the potential of these infections to have a major impact on the bulk milk somatic cell count.

Materials and Methods: Records of Quality Milk Production Services (QMPS) from January 1 1990 until March 1st 2007 were analyzed. An approximate total of 1,9 million individual cow or quarters sample records were available in the dataset. Composite milk samples were collected by QMPS personnel and culture in one of the four regional laboratories (for more details on the QMPS program see www.qmps.vet.cornell.edu). SCC data were obtained from DHIA records and were matched to the culture results by closest test day relative to the day of milk sampling for bacteriology. Only records from cows where SCC data were available, and where only one organism was grown in bacterial culture of the milk sample (or culture negative) were used for analysis. A total of 352,614 records qualified for this study. Descriptive analyses were performed. For each observation the percent contribution of the cow to the herd’s estimated bulk milk SCC (BMSCC) was calculated. For each pathogen, total contribution to bulk tank SCC during a whole herd culture was calculated. A mixed model linear regression of linear score was performed. Herd was treated as a random effect, days in milk (categorized by month), lactation number (heifers versus cows), pathogen code an all possible interactions were included as fixed effects. Statistical significance was set at P<.05.

Results: Descriptive statistics of the most prevalent pathogens are shown in Table 1. The results showed three distinct populations: negative cultures, CNS & C. bovis showing a moderate increase in SCC and S. agalactiae, Strep spp. & S. aureus showing an important increase in SCC. Bulk milk SCC is plotted against the total percentage contribution of CNS infected quarters in Figure 1. Approximately 2% of herds would potentially have high SCC problems (>400 Bulk Milk SCC) ‘due to’ CNS. The mixed model analysis showed an important contribution of all pathogens to the total linear score variability. Least square means of the most prevalent pathogens by month in milk are shown in figure 2. Infection with CNS showed a larger increase in linear score in heifers compared to cows.

Conclusions: Intramammary infection with CNS resulted in a moderate increase in SCC. CNS infections are relatively more important in heifers relative to cows. Very few herds would have an important increase in bulk milk SCC that could be attributed mostly to CNS infections.

References:

Table 1. Pathogen distribution and their associated linear score and SCC in heifers and cows.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Heifers</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LS (SE)</td>
</tr>
<tr>
<td>Negative</td>
<td>54947</td>
<td>2.29 (1.69)</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>667</td>
<td>4.85 (2.14)</td>
</tr>
<tr>
<td>Strep spp.</td>
<td>2623</td>
<td>4.64 (2.39)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2756</td>
<td>4.63 (1.88)</td>
</tr>
<tr>
<td>C. bovis</td>
<td>2803</td>
<td>3.01 (1.56)</td>
</tr>
<tr>
<td>CNS</td>
<td>13926</td>
<td>3.06 (1.57)</td>
</tr>
</tbody>
</table>

Figure 1. Bulk milk SCC versus contribution of SCC to bulk milk of CNS infected cows. CNS infection (>5%) is “responsible” for bulk milk SCC over 400,000 in 86 (~2%) herds (shaded).

Figure 2. Least square means linear score by pathogen for heifers (left) and cows (right).
SESSION 2: IDENTIFICATION AND DIFFERENTIATION

003 The Quest for the Perfect Test: Phenotypic Versus Genotypic Identification of CNS

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Introduction: In many modern dairy herds, Coagulase-negative staphylococci (CNS) are a frequent cause of bovine mastitis. In North American dairy herds, it is quite common for CNS to be recovered from about 15 to 20% of cases of subclinical and clinical mastitis. It is well known that CNS are the most common pathogens recovered from heifer mastitis. The genus Staphylococcus contains at least 35 species and 17 subspecies and it is conceivable that under the right circumstances most of these organisms could be capable of causing bovine mastitis. In spite of the high incidence of intramammary infections associated with CNS, species specific control programs are rare. This paper discusses the utility of phenotypic identification systems for CNS in light of evolving systems of genotypic identification.

Microbiological Examination of Milk Samples: Veterinarians and mastitis researchers are generally in agreement that microbiological examination of milk samples is vital for implementation of effective mastitis control. However, North American dairy farmers do not seem to feel the same urgency for pathogen specific mastitis diagnoses. Only 13% of Wisconsin dairy producers reported that they submit milk samples from all clinical cases and 51% indicated that they rarely submitted any milk samples for microbiological analysis. Rudimentary farm based culturing systems that use selective medias to differentiate Gram-positive from Gram negative organisms are used by 15% of Wisconsin dairy farms but only 41% of those farmers used the results to direct treatment programs (2006, unpublished). Mastitis control programs have been developed for major contagious and environmental pathogens but few specific recommendations exist for control of CNS. The efficacy of treatment of CNS infection is generally high but between farm variation has been observed. If differences in treatment outcomes of CNS could be accounted for by variation in species, precise identification may be useful, but existing phenotypic systems are probably sufficiently accurate for this level of decision making and at least 1 study reported no differences in bacterial cure rates of CNS based on genotypic identification.

Accuracy of Methods Used to Identify CNS: Historically, the identification of mastitis pathogens has been based on conventional microbiological procedures which include growth on various medias, observation of colony morphology, hemolysis patterns, Gram staining characteristics, agglutination tests and use of biochemical profiles. A number of commercial identification kits and diagnostic schemes have been developed to speciate Staphylococci. Mastitis researchers have performed a plethora of studies to evaluate the accuracy of these tests and most phenotypic systems are considered to accurately identify >80% of staphylococci. In general most of these tests have been validated using human isolates and perform consistently but tremendous variation in identification among tests may be experienced. We observed a typical outcome when we evaluated the use of 2 commercial systems for identification of 54 Staphylococci obtained from cases of mastitis. All ATCC QC isolates (n=8) used in our study were properly identified by both testing systems but considerable divergence in identification of clinical isolates were seen among tests. Satisfactory agreement (Kappa >0.87) were achieved at the genus and species levels for API Staph but low agreement was seen for the BBL Crystal Gram+ system (kappa of 0.25 for
species identification). These results were not unexpected because the BBL Crystal database does not include the most commonly reported CNS species of chromogenes, hyicus and simulans. While this type of study confirms that differences occur among phenotypic identification systems, virtually no differences in treatment or control programs would have been recommended based on the lack of precision in bacterial identification. The consistent use of a phenotypic identification system that contains an adequate number of veterinary isolates in the diagnostic algorithm should have sufficient precision for mastitis control programs and most research needs. While an argument could be made that precise species identification of CNS is necessary for research on antimicrobial resistance, few mastitis specific breakpoints are currently available and breakpoints for Staphylococci do not currently extend below the genus level.

Conclusions:
A compelling argument for the necessity of enhanced precision in identification of CNS has not been identified. Control programs for CNS are not based upon species level identification and current research has not identified differences in treatment outcomes based on genotypic identification. CNS are widely distributed in nature and their ability to cause mastitis is undisputed. Research on these pathogens may be limited by the failure to speciate but until a compelling reason for precise identification is made the use of a standard phenotypic method is probably sufficient.

References:
Species identification of CNS: Genotyping as the Gold standard

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Introduction: In some parts of the world, coagulase negative staphylococci (CNS) are increasingly seen as an important cause of mastitis. Presence of CNS has been associated with increased somatic cell counts, clinical mastitis, decreased production and reduced herd life. On the other hand, CNS may have a beneficial effect, mostly if they protect the udder from infection with major pathogens. Not all CNS species are equally likely to have negative or positive effects. For example, a protective effect has been described for Staphylococcus chromogenes, while the closely related species Staphylococcus hyicus may not have this effect. Whether transmission mechanisms of CNS are species specific is largely unknown. Knowledge of the impact and epidemiology of CNS infections is necessary to evaluate which infection control measures are feasible and economically viable. For accurate measurement of the impact, sources and transmission mechanisms of individual CNS species, accurate identification of CNS species is a prerequisite. In this contribution, merits of phenotypic and genotypic methods for CNS species identification are compared.

Phenotypic Methods: Phenotypic methods are based on the expression of a characteristics by isolates, as opposed to the detection of DNA encoding those characteristics. Phenotypic traits include morphology, growth characteristics, ability to metabolize substrates, antimicrobial resistance, etc. Phenotypic methods for species identification of Staphylococci include API20Staph, API ID 32 Staph, Staph-Zym, the Vitek system and other combinations of biochemical tests, which may not be available in commercial formats. Phenotypic methods may suffer from lack of reproducibility or discriminatory power, unless the number of tests included is large. Phenotypic methods are often touted as cheaper than genotypic methods. Whether or not this is true depends in part on turnover, which affects opportunities and needs for automation, and the risk of reagent expiration. In addition to the price of the test, the costs of additional testing or inaccurate test results must be considered when comparing phenotypic and genotypic methods.

Genotypic Methods: Genotypic methods use DNA as the basis for identification. Many genotypic methods are used for strain typing, i.e. for differentiation of isolates at the subspecies level. Genotypic methods can also be used for identification at species level. Examples include ribotyping, tDNA-PCR, and DNA sequencing. The most common target for DNA-sequencing is the 16S rDNA gene. This gene has been used for characterization of polymicrobial populations from a variety of ecosystems, ranging from seawater to the bovine rumen and gastrointestinal tract. 16S rDNA sequencing has begun to replace DNA-DNA hybridisation as reference method. Several bacterial housekeeping genes are also used for species identification. For CNS, identification systems based on sodA, cpn60, and rpoB have been developed. In general, genotypic methods have higher discriminatory power and reproducibility than phenotypic methods. When genotypic methods were first developed, they tended to be more labour intensive or expensive than phenotypic methods. While some methods, such as automated ribotyping, are still costly, other methods are not necessarily more expensive than phenotypic methods.

Databases: For interpretation of results from phenotypic or genotypic assays, comparison with reference data is necessary. New species of CNS continue to be identified. Recent examples include Staphylococcus nepalensis, which was first isolated from a goat in the Himalayas and identified as a new species in 2005, and Staphylococcus fleuretti, which was first named in 2000. Both species have since been isolated from heifer milk in Europe. Most
Phenotypic methods were developed for use in human medicine. The prevalence of CNS species differs between humans and animal species. CNS species that are important for veterinary medicine may not be highly relevant for human medicine and accurate identification of species of veterinary importance may not be a priority during test development. Within a bacterial species, strains isolated from animals may differ from those isolated from humans. The phenotypic profile used to identify human isolates to the species level may not accurately identify animal isolates. Phenotypic systems for routine diagnostic use cannot be updated every time a new bacterial species is identified or strain level differences between isolates from different host species are recognized. With the use of automated on-line databases such as GenBank, it is extremely easy to add new strains or species to a reference DNA database shortly after their detection. Finally, use of DNA-sequence based identification provides a quantitative measure, down to the last base pair of the genetic code, of the certainty with which an isolate has been identified. For other genotypic methods, such as automated ribotyping and tDNA-PCR, some of the limitations of phenotypic methods apply, i.e. similarity coefficients can be calculated but there is no universally meaningful quantitative measure of the genetic relatedness of isolates, and reference databases are not updated with the same speed as DNA-sequence based reference libraries.

Discussion: Efforts are underway to compare species identification of bovine CNS by genotypic and phenotypic methods. Some comparisons will be presented elsewhere in these proceedings. Using DNA-sequencing, almost all isolates can be characterized, and over 99% can be identified. So far, other genotypic methods and phenotypic methods have lower typeability and accuracy, which may manifest in missing, incorrect, or ambiguous results. The choice of typing methods should be determined by a number of considerations, such as the goal of species identification, speed, ease of use, cost, availability of equipment and trained personnel, etc. In the case of CNS identification in the context of mastitis control, the best method would be a fast, simple and cheap method that provides a relevant level of differentiation. The relevant level of differentiation is yet to be established, because species level information is currently barely available. Once the most relevant species have been identified, simplified phenotypic or genotypic methods targeting this subset of CNS may be of value for routine diagnostics.

Conclusion: DNA-sequence based species identification of CNS is currently the most accurate species identification method available because it has the largest reference database, and because a universally meaningful quantitative measure of homology with known species is determined. DNA sequence based species identification should therefore be considered the Gold standard. Which level of accuracy and which characteristics of CNS isolates are relevant for management and control of CNS mastitis in dairy herds is unknown. Once sources, transmission mechanisms and impact of different CNS species have been identified through use of epidemiological data and accurate species identification methods, appropriate methods for use in research and diagnostic laboratories can be selected.
SESSION 3: ANTIBIOTIC RESISTANCE/ VIRULENCE AND PERSISTENCE

005 Antibiotic resistance in Coagulase Negative Staphylococci: an emerging problem?

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Introduction: Although the importance of coagulase negative staphylococci (CNS) in relation to mastitis in dairy cattle is not yet fully understood, as a result of control measures both environmental streptococci and CNS are more frequently isolated from cases of mastitis \(^1,2\). In the Netherlands the prevalence of coagulase negative staphylococci isolated from milk samples of subclinical mastitis cases from Dutch dairy cows increased from 16.2% in 1999 to 42.2% in 2004\(^2\). In samples of clinical mastitis cases these percentages were respectively 7.3% and 14.1%. CNS is mainly associated with sub-clinical infections \(^3\). In this paper the susceptibility of CNS to antibiotics and the emergence of resistance is discussed.

Materials and Methods: Since 2002, in the Netherlands annually approximately 100 CNS strains isolated from bovine cases of mastitis by the Animal Health Service in Deventer are examined for susceptibility to a panel of antibiotics (see Table1. and Fig. 1) used in veterinary medicine. MICs are determined with the broth micro dilution methods according to CLSI guideline M31-A2 using Sensititre® microtitre trays manufactured by TREK Diagnostic Systems (Basingstoke, UK). Oxacillin resistance was confirmed by PCR aimed at the \(\text{mecA}\) gene.

Results and Discussion. Resistance to beta-lactam antibiotics occurred frequently in CNS (Table 1, Fig 1). Although the main resistance mechanism was production of penicillinases, throughout the years approximately 5% of the isolates tested were oxacillin resistant due to the presence of the \(\text{mecA}\)-gene. Although the measured MIC values indicate that \(\text{MecA}\)-positive isolates were still susceptible to amoxicillin-clavulanic acid and cephalothin based on the CLSI MIC-breakpoints, these isolates were classified resistant to these antibiotics because \(\text{mecA}\)-positive staphylococci are by definition resistant to all beta-lactam antibiotics including all cefalosporins available in veterinary medicine. Differences in penicillin resistance level before and after 2004 can be explained by the fact that in 2002 and 2003 no beta-lactamase test was performed to confirm penicillin resistance. The MIC data show that the CLSI breakpoints are not entirely adequate to detect both penicillinase and \(\text{mecA}\)-derived beta-lactam resistance in CNS. \(\text{MecA}\) is horizontally transferable. Widespread use of beta-lactams in treatment or prevention of mastitis may induce this transfer to \(S.\) \(\text{aureus}\) and create MRSA in dairy cattle. Although this phenomenon has not been described to have occurred in cattle, it has very likely been the cause for the MRSA clone that is currently spreading in food animal production in Europe\(^4\). Therefore, also in dairy cattle to control mastitis antibiotics should be used restrictively and rationally.

Macrolides and lincosamides are frequently used for treatment of mastitis in The Netherlands. Resistance to these antibiotics occurred in various proportions. Resistance to erythromycin and lincomycin/clindamycin seems to have decreased slightly. For the lincosamides this obvious decrease could partly be explained by the fact that in 2002 and 2003 lincomycin was used and afterwards clindamycin. Clindamycin is more potent that lincomycin. Moreover, CLSI breakpoints are only established for clindamycin which indicates that using lincomycin will result in an overestimate of lincosamide resistance. Pirlimycin resistance levels varied from 5 to 10%. All isolates resistant to erythromycin were tested for inducible clindamycin resistance by double disk test. Resistance to tetracycline, streptomycin, kanamycin and
trimethoprim-sulfa occurred annually in levels around 10% of the isolates tested. Resistance to antibiotics observed in The Netherlands will be discussed in relation to published data on CNS susceptibility.

**Conclusion.** Based on broth microdilution testing, in Dutch CNS isolates resistance to beta-lactams is very commonly based on the production of narrow spectrum beta-lactamases. Approximately 5% of the isolates are oxacillin resistant based on the presence of the mecA gene. For both resistance mechanisms additional tests (beta-lactamase test and PCR) improve the quality of the susceptibility test results substantially. MecA is horizontally transferable. Widespread use of beta-lactams in treatment or prevention of mastitis may induce this transfer to S. aureus and create MRSA in dairy cattle. Resistance to macrolides and lincosamides occurred in various proportions. Resistance to tetracycline, streptomycin, kanamycin and trimethoprim-sulfa varied annually in levels around 10% of the isolates tested.

**References:**
006 The coagulase-negative staphylococci – not so different from Staphylococcus aureus?

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Introduction
Mastitis causing staphylococci are usually divided into two groups: Staphylococcus aureus and other staphylococci, commonly called as coagulase-negative staphylococci, CNS. CNS are considered as a uniform group, although they comprise 39 characterized species. Some of them are in fact coagulase-positive. The list of CNS species most often isolated from mastitis is much shorter, and a few species seem to dominate. As mastitis causing agents, CNS are always separated from S. aureus, but are they so different?

Bovine CNS species and their characteristics
In CNS isolated from bovine mastitis, two species, S. chromogenes and S. simulans, dominate (Jarp, 1991; Waage et al., 1999; Taponen et al., 2006). S. epidermidis and S. hyicus are other species often reported (Waage et al., 1999). S. aureus is considered contagious, the main source being infected mammary quarters. CNS are not considered so contagious, but not much data on this is available. Humans and cows do not generally share same S. aureus strains. Thorberg et al. (2006) found the same S. epidermidis strains both from cows’ milk and milker’s hands, indicating that S. epidemidis strains causing mastitis may originate from humans. Our preliminary results show that the same strains of S. chromogenes and S. simulans can be found both from bovine extramammary sites and mastitis (Taponen et al., unpublished).

Clinical significance of CNS mastitis
S. aureus is able to cause clinical mastitis with severe local and systemic signs, but very often also subclinical mastitis which remains persistent. CNS have been regarded as minor pathogens, which mostly infect heifers around calving, do not cause clinical signs, only slightly increase the somatic cell count, and disappear soon after parturition. Very few studies on the clinical characteristics of mastitis caused by CNS are available. In the few studies where clinical signs of CNS mastitis have been reported, mastitis most often has been subclinical or mildly clinical (Jarp, 1991; Taponen et al., 2006). Only mild local signs usually can be seen such as slight swelling and changes in the milk appearance. Systemic signs, like fever have been rare. One study (Bleul et al., 2006) reported on three cases of toxic mastitis caused by staphylococci other than S. aureus. In that study, coagulase production of the isolates was not tested. S. aureus usually increases milk somatic cell count (SCC) substantially, but in CNS mastitis SCC typically remains much lower. In a meta-analysis of Djabri et al. (2002), the geometric mean SCC in the CNS infected quarters was 138 000 cells/ml and in the S. aureus infected quarters 357 000 cells/ml.

Mastitis caused by S. aureus is known to respond poorly to antimicrobial treatment. The reported cure rates vary largely, from 15% to 85%, depending on many variables like lactation number, SCC before treatment and beta-lactamase production of the causing isolate (Barkema et al., 2006). Not many treatment studies which separately report results for quarters infected by CNS have been published. Based on those which are available, mastitis caused by CNS seems to respond well to antimicrobial treatment; the bacteriological cure rates have been 80-90% (McDougall, 1998; Taponen et al., 2006). In most studies, penicillin G has been used in the treatment and the isolates were tested in vitro for susceptibility for
penicillin. Elimination rates for mastitis caused by penicillin-resistant CNS seem to be somewhat lower (Taponen et al., 2006).

S. aureus in known of its ability to persistently infect the bovine udder. New evidence shows that CNS infections can also persist in the mammary gland (Aarestrup and Jensen; 1997, Taponen et al. 2006). When 228 udder quarters of 82 cows on one farm were followed monthly throughout lactation, it was shown that about half of the CNS infections persisted (Taponen et al., unpublished). In a similar study from Norway (Merk et al., unpublished) where udder quarters of cows on four farms were followed monthly, results were very similar. It can be concluded that many CNS infections indeed are able to persist throughout the lactation. The clinical significance of persistent CNS infections is not likely to be as high as that of S. aureus infections, due to the lower contagiousness and less damage to the mammary gland.

**Virulence factors**

Virulence factors of S. aureus and CNS have been investigated both by measuring phenotypic expression of products assumed to be associated with virulence and by screening of genes encoding these. Various virulence factors, like production of haemolysins, leucocidins, exfoliative toxins, enterotoxins, toxic-shock syndrome toxin, and ability of slime and biofilm formation have been found in S. aureus strains isolated from bovine mastitis (Cucarella et al., 2004; Zecconi et al., 2006). Only few studies have been focused on virulence factors from CNS isolated from mastitis. Some strains of S. aureus and CNS from bovine mastitis have shown to invade bovine mammary gland epithelial cells in cell cultures (Anaya-López, 2006). Majority of CNS isolated from caprine mastitis have been found to produce at least one type of haemolysin, DNAse, and elastase (Bedini-Madani et al., 1998; Da Silva et al., 2005). Kuroishi et al. (2003) found that a high percentage of both S. aureus and CNS from bovine subclinical, chronic or acute mastitis produced staphylococcal enterotoxins and/or toxic shock syndrome toxin-1. Various virulence factors of S. aureus have been compared with clinical characteristics of mastitis, but no certain virulence factor has been strongly associated with the severity of mastitis (Haveri et al., unpublished). On the contrary it has been shown that biofilm producing S. aureus strain decreased severity of mastitis but increased the colonization capacity in the mammary gland (Cucarella et al., 2004). Biofilm associated proteins can be found among bovine mastitis isolates, including S. aureus, S. epidermidis, S. chromogenes, S. hyicus, and S. xylosus (Cucarella et al., 2004). Oliveira et al. (2006) found 6 out of 16 S. aureus and 6 out of 16 S. epidermidis isolates from subclinical mastitis phenotypically positive for biofilm production. Based on the literature available, it can be concluded that CNS are clearly less pathogenic than S. aureus, however some species may be more harmful than others. More research is needed to confirm this.

**References**


007 Prevalence of coagulase negative staphylococci on dairy farms in The Netherlands

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Introduction: Coagulase-negative staphylococci (CNS) are considered to be minor mastitis pathogens. However, CNS are isolated from cases of subclinical and clinical mastitis cases, and also from teat canals, teat skin and teat ducts (1,2). Additionally CNS are the predominant pathogens in heifer mastitis (4,5). The aim of this study was to analyse the prevalence of intramammary infection (IMI) with CNS species in Dutch dairy cows and to study possible risk factors associated with prevalence of CNS IMI.

Materials and Methods: In this survey, 49 herds were randomly selected stratified per province. Herds were included that had at least 40 lactating cows and participated in the Dutch milk recording system. Milk samples were collected from all quarters of a selection of cows, based on cow somatic cell count (SCC) of the last milk recording before the farm visit. Two groups of cows were selected: 1) a high SCC (HSCC) group that consisted of all cows and heifers with a SCC >250,000 cells/ml and >150,000 cells/ml, respectively, and 2) a low SCC (LSCC) group that consisted of approximately 25% of cows and heifers with a SCC ≤250,000 cells/ml and ≤150,000 cells/ml, respectively. Quarter foremilk samples were collected aseptically by personnel of the Animal Health Service (AHS). Bacteriological culturing was performed according to the NMC protocols (3). A selection of CNS was tested with the API Test, (BioMerieux). The Apilab software was used to calculate the probability of the identification result in a range of 10 to 100%. Possible tests were suggested when needed for the delineation of species.

A questionnaire was conducted to obtain management information of the farms such as housing facilities, milking procedures, feeding and treatment regimes. The association between these management factors and prevalence of CNS IMI was determined using a generalized linear model. All variables with a P-value < 0.25 in a univariable analysis were offered to the multivariable analysis. The final model was constructed by means of a backwards elimination method, checking for possible confounders and interactions. Variables with P-value > 0.05 (T-test) were removed from the model. All possible two-way interactions between remaining significant variables were investigated. Normality of the data was checked on the residuals and the fit of the model was given by the adjusted R².

Results: Bacterial growth occurred in 37.7% of the 2174 collected milk samples from HSCC cows and in 21.1% of the 2046 collected milk samples from LSCC cows (P<0.0001). Coagulase-negative staphylococci were the most frequently isolated group of bacteria in both the HSCC and the LSCC group, 14.6% and 10.4% respectively. The difference in prevalence of CNS IMI between these two groups was significant (P<0.05). The prevalence of mastitis pathogens differed among herds. Coagulase-negative staphylococci were found in quarter milk samples of all farms. Distribution of CNS differed considerably among parity groups. 49.4% of the heifers had one or more quarters shedding CNS, this was 30.9 and 33.1% in second and third parity cows, respectively (Figure 1). From the 530 isolated CNS, 158 were offered for identification with the API test. In total 14 species were identified: the predominant CNS species was Staph. chromogenes (30.3%) followed by Staph. epidermidis (12.9%) and Staph. simulans (11.0%). Staphylococcus haemolyticus was significantly more prevalent in
the LSCC group in comparison with the HSCC group. The prevalence of the identified species did not differ between the parity groups. Quarters with a CNS IMI had a geometric mean quarter SCC of 109,000 cells/ml, which was almost twice as high of the culture-negative quarters which had a quarter SCC of 58,000 cells/ml ($P<0.05$). *Staphylococcus epidermidis* and *Staph. simulans* had significant higher SCC in the HSCC in comparison with the LSCC group ($P<0.05$). The SCC of the other species did not differ between the two groups.

After the univariable analysis of the questionnaire only three variables remained, but the model showed a good fit to the data (adjusted $R^2 = 0.89$). Factors that were associated with an increased prevalence of CNS IMI were using ditchwater instead of tap water as drinking water ($P=0.02$), pasturing the cows during summer instead of keeping the cows indoors ($P=0.008$) and using one instead of two dry cow groups of ($P=0.03$). In the final model production level, post-milking teat disinfection, dry cow therapy, housing facilities around calving and type of bedding did not attribute to the difference in the prevalence of CNS IMI between the herds studied.

**Conclusion:** In this study, the prevalence of CNS in The Netherlands was estimated on 49 farms with at least 40 lactating cows. The farms were randomly selected from the national database. In total, 4220 quarter milk samples were collected; the predominant subclinical mastitis pathogens were CNS. Fourteen species of CNS were identified; the predominant species was *Staph. chromogenes* (30.3%) followed by *Staph. epidermidis* (12.9%) and *Staph. simulans* (11.0%). The prevalence of CNS was higher in heifers in comparison with older cows. Geometric mean quarter SCC of CNS-positive quarters was 109,000 cells/ml, which twice as high in compared to culture-negative quarters (58,000 cells/ml). The multivariable analysis on the questionnaire of management information showed that using ditchwater, pasturing during the summer and using one instead of two groups of dry cows increased the prevalence of CNS IMI.

**References:**

**Figure 1:** Parity distribution of intramammary infection with coagulase negative staphylococci.
Role of coagulase-negative staphylococci in human disease.

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Introduction: Coagulase-negative staphylococci (CNS) are normal inhabitants of human skin and mucous membranes. They have long been dismissed as culture contaminants, even in type 1 samples (obtained from a normally sterile place by needle aspiration or surgery), but now the potentially important role of CNS as pathogens and their increasing incidence has been recognized. The distinction between clinically significant, pathogenic strains and contaminating strains remains a major problem. We will give an overview of the medical literature on CNS, with special focus on the history, virulence factors, antibiotic resistance, epidemiology and specific infections caused by CNS in men.

Results: In 1884 S. albus was described as an avirulent staphylococcus by Rodenbach. Only in 1958 the first report on the potential pathogenicity of CNS in patients with septicaemia was published. Later on casuistic reports of CNS endocarditis, wound and urinary tract infections (UTI) were published (6). Since the 1970s CNS are recognised as etiologic agents of a wide variety of infections. Patients are usually immunocompromised, with indwelling or implanted foreign bodies. In the immunocompetent host CNS endocarditis and UTI with S. saprophyticus are generally accepted CNS infections. Only 16 of all CNS species have been found in specimens of human origin. Accurate identification of CNS isolates to species level is difficult to perform, expensive and seldom necessary for good patient management.

Already in 1972 the “slime” production of S. epidermidis was noted as an important factor in the pathogenesis (5). This biofilm formation is the most important virulence factor of S. epidermidis. The polysaccharide intercellular protein PIA, encoded by the icaADBC locus plays an important role in biofilm formation (13). But PIA negative, biofilm positive strains have been described (9). The virulence genes icaADBC, mecA and insertion sequence (IS) 256 are significantly more prevalent in strains from hospitalized patients than in healthy individuals. However, their prevalence does not differ between commensal and invasive strains in a bone marrow transplant unit (11).

In nosocomial strains approximately 80% of the strains is methicillin resistant and multiresistant (resistant to macrolides, lincosamides, aminoglycosides, tetracycline, fluoroquinolones). Most resistances are found in S. epidermidis and S. haemolyticus. Community acquired strains are mostly susceptible (15). However, in a recent study in medical students, prior to their beginning of clinical practice, methicillin resistance was found in 23.5% and multidrug resistance in 13% of CNS strains (4). CNS were the first organisms in which glycopeptides resistance was recognized (1). Resistance to linezolid is also emerging. In surveillance studies <0.1% of CNS are linezolid resistant, however in one institution 4% of CNS were found to be linezolid resistant. These resistant strains showed genetic relatedness (8).

In another study the majority of clinical strains, analysed by multilocus sequence typing belong to one clone (ST27) (7). These findings suggest that the same strict hand hygiene measures as for methicillin resistant S. aureus should be followed for multiresistant CNS strains and if clonal spread is proven one should also consider more stringent contact isolation precautions.

S. saprophyticus is the second most frequent causative organism of uncomplicated UTI in young women. Other CNS are usually isolated from hospitalized, elderly patients with urinary catheters (10). In native valve endocarditis CNS account for 5% of all episodes. Rates of heart failure and mortality were found to be similar between patients with CNS and S. aureus native valve endocarditis (2), which is in contrast with the believed indolent clinical course of CNS endocarditis. Infections of prosthetic valves are caused by CNS in 40-50% of
cases (5). In a US nation wide surveillance study CNS account for 31% (14), in Flanders for 25.9% of all nosocomial blood stream infections. Forty-eight percent of these concerned catheter related sepsis, 32% sepsis of unknown origin and 20% were associated with another infection or an invasive manipulation (12). Of course the intensity of surveillance and the criteria for defining true bacteraemia may influence these prevalences. There is more and more evidence that the mucosa, instead of the skin, is the likely source of CNS bacteraemia in cancer patients (3). Other important infections due to CNS include central nervous system shunt infections, endophthalmitis after penetrating eye injury or vitrectomy, surgical site infections, peritonitis in patients with continuous ambulatory peritoneal dialysis and low grade foreign body infections (aseptic loosening of hip prostheses, late-onset endophthalmitis after cataract surgery). CNS are rarely associated with mastitis in humans. *S. lugdunensis* is more pathogenic than other CNS (expression of a clumping factor, DNase, haemolysin) (13). *S. lugdunensis* infections are rare and tend to resemble *S. aureus* infections.

**Conclusion:** The increased incidence of CNS infections is clearly the result of medical progress and mainly due to the use of invasive and indwelling medical devices. CNS are now a major cause of nosocomial infections (in particular nosocomial bacteraemia). Many interesting questions concerning the epidemiology, pathogenesis and antimicrobial resistance are not yet fully solved.

**References:**


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(7) Kozitskaya et al., 2005, Clonal analysis of *Staphylococcus epidermidis* isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. J clin microbiol, 43: 4751–4757

(8) Potoski et al., 2006, Epidemiological profile of linezolid-resistant coagulate-negative staphylococci. CID, 43: 165-171


(10) Raz et al., 2005, Who Are You—*Staphylococcus saprophyticus*? CID, 40: 896-898

(11) Rohde et al., 2004, Detection of virulence-associated genes not useful for discriminating between invasive and commensal *Staphylococcus epidermidis* strains from a bone marrow transplant unit. J clin microbiol, 42: 5614–5619


(13) von Eiff et al., 2002, Pathogenesis of infections due to coagulate negative staphylococci. Lancet Infect Dis, 2: 677–85


SESSION: POSTER PRESENTATIONS

P001 Comparison of tuf gene sequencing and biochemical typing (Staph-Zym) for species identification of coagulase negative staphylococci from bovine mastitis

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Introduction: The understanding of the pathogenesis and control of bovine mastitis caused by coagulase negative staphylococci (CNS) is complicated by the heterogeneity of this group of bacteria. In order to identify better tools for diagnosis of different species of CNS from bovine mastitis, the aim of the present study was to compare tuf gene sequencing with a commercial biochemical typing system, Staph-Zym.

Materials and Methods: Twenty-four reference strains (CCUG, ATCC and NCTC) of 17 different species of Staphylococci were used (Table 1). In addition, 62 and 20 CNS isolates from clinical and sub-clinical cases of bovine mastitis, respectively, were analysed. Genotyping was performed according to Heikens et al. (2005)2. Phenotyping was performed with the Staph-Zym (A/S Rosco, Taastrup, Denmark) system according to the manufacturers instruction, including supplementary tests as recommended. In addition to the sequence information obtained for the reference strains included, tuf sequences deposited in GenBank2,3 were also used when assigning a species name to the mastitic isolates. If an isolate could not be defined by its tuf sequence, part of the 16S rRNA gene was sequenced, and a species name was assigned after comparison with 16S rRNA sequences deposited in GenBank.

Results: The number of CNS species identified using tuf gene sequencing, and the agreement with Staph-Zym results for the 82 bovine isolates are presented in Table 2. In total, tuf sequencing identified nine different species of CNS. Overall, the most common species were S. chromogenes (27%), and S. simulans (24%). S. chromogenes (29%) was also the most common species among the clinical isolates, while S. epidermidis (30%) was the most common species among the sub-clinical cases. Compared to tuf gene sequencing, Staph-Zym mis-identified 32 (39%) of the isolates. The agreement between the two tests was better for S. simulans than for S. chromogenes. Use of Staph-Zym, supplementary tests was required in 27 cases of which S. chromogenes accounted for 67%. In 9 cases Staph-Zym could not identify the species, and four of those were S. chromogenes.

Conclusion: The present study agrees with other studies1,2 in that genotypic methods are superior to phenotypic assays for identification of bovine CNS. The high number of misidentified isolates and the frequent need of additional tests when using Staph-Zym makes this method both inaccurate, and time consuming.

References:
Table 1: Reference strains of the genus Staphylococci used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>CCUG / NCTC / ATCC</th>
<th>Species</th>
<th>CCUG / NCTC / ATCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (n=2)</td>
<td>CCUG 46176</td>
<td>S. intermedius (n=3)</td>
<td>CCUG 6520</td>
</tr>
<tr>
<td></td>
<td>CCUG 41582 (NCTC 8325)</td>
<td></td>
<td>ATCC 51874</td>
</tr>
<tr>
<td>S. capitis (n=1)</td>
<td>CCUG 7326 (ATCC 27840)</td>
<td>S. pulvereri (n=1)</td>
<td>CCUG 41685</td>
</tr>
<tr>
<td>S. chromogenes (n=1)</td>
<td>CCUG 4319</td>
<td>S. saprophyticus(n=1)</td>
<td>CCUG 3706</td>
</tr>
<tr>
<td>S. cohnii (n=1)</td>
<td>CCUG 35144</td>
<td>S. schleiferi (n=2)</td>
<td>ATCC 49545</td>
</tr>
<tr>
<td>S. epidermidis (n=2)</td>
<td>CCUG 46178</td>
<td>S. sciuri (n=1)</td>
<td>CCUG 15598</td>
</tr>
<tr>
<td></td>
<td>CCUG 21989</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. gallinarum (n=1)</td>
<td>CCUG 28809</td>
<td>S. simulans (n=2)</td>
<td>CCUG 46177</td>
</tr>
<tr>
<td>S. haemolyticus (n=1)</td>
<td>CCUG 7323</td>
<td></td>
<td>CCUG 7327</td>
</tr>
<tr>
<td>S. hominis (n=1)</td>
<td>CCUG 35516</td>
<td>S. warneri (n=1)</td>
<td>CCUG 44859</td>
</tr>
<tr>
<td>S. hyicus (n=2)</td>
<td>CCUG 5509</td>
<td>S. xylosus (n=1)</td>
<td>CCUG 7324</td>
</tr>
<tr>
<td></td>
<td>CCUG 15602 (ATCC 11249)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 NCTC = National Collection of Type Cultures, Salisbury, UK; CCUG = Culture Collection, University of Göteborg, Göteborg, Sweden; ATCC = American Type Culture Collection, Manassa, USA.

Table 2: Species identification, using tuf gene sequencing, of 82 CNS strains isolated from cases of bovine mastitis, and number (%) of those isolates correctly identified using Staph-Zym.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Number of isolates correctly identified using Staph-Zym</th>
<th>Misjudged by Staph-Zym as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
<td>No (%)</td>
<td>S. cohnii</td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>22</td>
<td>13 (59)</td>
<td>9 (41)</td>
</tr>
<tr>
<td>S. simulans</td>
<td>20</td>
<td>17 (85)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>12 (2\a)</td>
<td>4 (33)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>10</td>
<td>8 (80)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>9</td>
<td>4 (44)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>S. warneri</td>
<td>4</td>
<td>2 (50)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>S. lentus</td>
<td>1 (\a)</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>50 (61)</td>
<td>32 (39)</td>
</tr>
</tbody>
</table>

\a Identified by 16s rRNA sequencing
**P002 Antimicrobial resistance of staphylococci isolated out of subclinical and clinical cases of mastitis**

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2Animal Health Care Flanders, Torhout, Belgium

**Introduction:** Mastitis is one of the most expensive diseases in dairy cattle. In contrast with subclinical mastitis, where practitioners can await the results of an antibiogram before treatment, cows suffering from clinical mastitis should be treated immediately. In the latter case, clinicians have to rely on their experience and monitoring data about pathogens and their antimicrobial susceptibility profiles. In the present project, the prevalence of several udder pathogens isolated from subclinical and clinical mastitis cases was assessed. In addition, the isolated staphylococci (coagulase-negative staphylococci and *Staphylococcus aureus*) underwent susceptibility testing for antimicrobial agents commonly applied for the control of udder infections.

**Materials and Methods:** Ten Flemish dairy farms with at least 40 lactating cows were continuously monitored during 3 years (2002-2004). In case of clinical mastitis (clinical symptoms and CMT test positive) milk samples of all infected quarters were taken, while in case of subclinical mastitis (composed somatic cell count in the last 3 months at least once higher than 200 000 cells/ml milk, based on the monthly results of the Flemish DHI-programme), all 4 quarters were sampled. In total 2119 quarters of 639 cows (507 with subclinical and 186 with clinical mastitis) were taken up in the study. Standard bacteriological procedures (NMC guidelines) were used for isolation of bacteria and susceptibility profiles were performed using the Kirby Bauer disk diffusion method using a standardized protocol for inoculation, incubation, and internal quality control.1 Reading of inhibition zones was performed by a semi-automated device (SIRscan 2000) and interpreted (Susceptible, Intermediate, Resistant) according to guidelines of the manufacturer of the tablets (Rosco, Denmark).

**Results:** In table 1 the prevalence of different pathogens on quarter level is calculated (n1 = number of sampled quarters; n2 = number of infected quarters) in subclinical and clinical mastitis. Figure 1 compares the resistance (+ intermediate resistance) levels of the CNS and *S. aureus*.

**Conclusion:** The relative contribution of CNS was greater in the clinical cases in this study (22.9%) compared with the official data of Flanders2 (16.3%). Most of the resistance traits were found in the CNS and not in *S. aureus*. Coagulase-negative staphylococci were frequently resistant for lincomycin, penicillin and tetracyclins, while only 15% and 12% of the *S. aureus* were resistant for penicillin and oxacillin respectively. The resistance levels of lincomycin, penicillin, tetracyclins and erythromycin were much higher for CNS than for *S. aureus*. These data illustrate the increasing clinical relevance of CNS in dairy herds in Flanders as their isolation in clinical mastitis cases is growing. The relatively high levels of antimicrobial resistance within this group of bacteria is another important point of concern.

This study was supported by the Belgian Federal Public Service of Public Health, Food Chain Security and Environment (Grant S-6136 & S 6166). Els Defré is acknowledged for technical assistance.

**References:**
1. Anonymous (1999). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standards. NCCLS


Figure 1: Comparison of resistance (including intermediate resistance) between CNS and S. aureus.

Table 1: Prevalence (absolute and relative) of different pathogens in the sampled (n1) and infected (n2) quarters of cows with subclinical (507) and clinical (186) mastitis.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Subclinical cases</th>
<th>Clinical cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n1 = 1879 (%)</td>
<td>n1 = 240 (%)</td>
</tr>
<tr>
<td></td>
<td>% (n2 = 1050)</td>
<td>% (n2 = 189)</td>
</tr>
<tr>
<td>CNS</td>
<td>342 (18,20)</td>
<td>55 (22,92)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>102 (5,43)</td>
<td>44 (18,33)</td>
</tr>
<tr>
<td>Streptococci</td>
<td>159 (8,46)</td>
<td>57 (23,75)</td>
</tr>
<tr>
<td>C. bovis</td>
<td>329 (17,51)</td>
<td>6 (2,50)</td>
</tr>
<tr>
<td>E. coli</td>
<td>46 (2,45)</td>
<td>27 (11,25)</td>
</tr>
<tr>
<td>Others</td>
<td>8 (0,43)</td>
<td>2 (0,83)</td>
</tr>
<tr>
<td>Polybacterial</td>
<td>209 (11,12)</td>
<td>10 (4,17)</td>
</tr>
<tr>
<td>Negative</td>
<td>829 (44,11)</td>
<td>51 (21,25)</td>
</tr>
</tbody>
</table>
P003 Prevalence of coagulase-negative *Staphylococcus* species from three Tennessee dairy research herds

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Introduction
Coagulase-negative *Staphylococcus* species, more commonly referred to as CNS, are isolated frequently from bovine milk. However, the importance of CNS intramammary infections has not been clearly delineated. The literature often refers to CNS as minor mastitis pathogens, which suggests that CNS are non-pathogenic or mildly pathogenic. The prevalence of CNS from various herds has been reported to range from 3% to 30% of quarters, and involve 27% to 55% of cows (1). The prevalence of CNS in mammary secretions of primigravid heifers during the prepartum period has been reported to be as high as 50% of mammary quarters (4). In three Tennessee dairy research herds, 22% of mammary quarters of lactating cows were infected with CNS (4). The last study that identified CNS from these three farms was reported over 15 years ago. At that time, 105 CNS isolates were identified by Vitek and API Staph-Trac and the prevalence was as follows: *Staphylococcus chromogenes* (40%), *S. simulans* (13.3%), *S. hyicus* (13.3%), *S. xylosus* (8.6%), *S. sciuri* (5.7%), *S. warneri* (4.7%), *S. epidermidis* (4.7%), *S. hominis* (3.8%), *S. haemolyticus* (3.8%), *S. capitis* (<1%), and *S. saprophyticus* (<1%) (2). The objective of the present study was to determine the prevalence of CNS from the same three dairy research herds of The University of Tennessee to determine changes that might have occurred over a 15 year time period.

Materials and Methods
Coagulase-negative *Staphylococcus* species isolated from quarter milk samples from three University of Tennessee research dairy herds were chosen randomly and identified to the species level by the API Staph System (bioMerieux Inc., Hazelwood, MO, USA). Additional tests to identify CNS included: β-glucosidase, β-glucuronidase, β-galactosidase and turanose. CNS were isolated from samples obtained from cows and heifers before calving, during early lactation, near drying off, during routine herd surveys, when cows were culled from the herd, and/or from cows with clinical mastitis. Only CNS from mammary quarters with > 5,000 colony forming units/ml were identified to the species level. Pulsed-field gel electrophoresis (PFGE) was performed on selected CNS isolates to determine if the same species was isolated over time. The restriction endonuclease Sma I (Roche Diagnostic Corporation, Indianapolis, IN, USA) was used following procedures described by McDougal et al. (3). Somatic cell counts (SCC) or somatic cell scores (SCS) were obtained from Dairy Herd Improvement Association (DHIA) records for selected cows. The SCC or SCS were from composite milk samples obtained ± 14 d of when CNS were isolated. These data were converted to log_{10} and analyzed using SAS v 9.1 (SAS Institute Inc., Cary, NC, USA). Average log_{10} SCC of uninfected cows was compared with the average log_{10} SCC of cows infected with CNS only.

Results
In 2005, CNS were isolated from approximately 11% of quarter milk samples from three dairy research herds. A total of 383 CNS were identified to the species level by API Staph System (bioMerieux Inc.) and supplemental tests. This represents approximately one third (383/1024) of all CNS isolated from these three farms in 2005. The prevalence from all three farms was as follows: *S. chromogenes* (48%), *S. hyicus* (26%), *S. epidermidis* (10%), *S. simulans* (7%), *S. warneri* (2%), *S. hominis* (2%), *S. saprophyticus* (1%), *S. xylosus* (1%), *S. haemolyticus* (<1%), *S. sciuri* (<1%), and *S. intermedius* (<1%). In two of the dairy farms
(DREC and ETREC), S. chromogenes (55% and 45%) and S. hyicus (30% and 21%), were the predominant species isolated. However, for the MTREC farm, the percentage of S. epidermidis was 24%, S. chromogenes 41% and S. hyicus 21%. In the DREC and ETREC dairies, S. epidermidis was only isolated in 5% and 3% of samples, respectively. Staphylococcus simulans was isolated more frequently from the ETREC dairy (18%) than the DREC (4%) or MTRES (4%) dairies.

A total of 160 CNS isolates (66 S. chromogenes, 33 S. hyicus, 39 S. epidermidis, 10 S. simulans, and 7 S. warneri) were analyzed by PFGE fingerprinting. These isolates were from 89 different cows/heifers with 41 having the same CNS isolated at intervals from 2 wk to 10 mo. PFGE patterns revealed that the majority (33/41) of isolates from the same mammary quarter had the same PFGE pattern indicating persistence of the infection over time. In a few instances, CNS isolated from the same cow on different occasions had a different PFGE pattern indicating a new infection by a different strain. Looking at all of the PFGE patterns for each of the CNS isolates, no common pulsotype was seen among the three farms.

We also evaluated composite cow SCC data obtained ± 14 d of when CNS were isolated. Average SCC from all three farms was 5.32 log_{10} somatic cells/ml of milk from cows in which CNS was the only bacteria isolated. Average SCC from all three farms was 4.90 log_{10} somatic cells/ml of milk from cows on the same farms with quarter milk samples negative for any bacteria. Thus, mammary quarters infected by CNS can contribute significantly (P<0.0001) to the number of somatic cells in milk.

Conclusions
The prevalence of CNS in these three dairy research herds has changed somewhat in the past 15 years. S. chromogenes continued to be the predominant species isolated; however, S. hyicus and S. epidermidis have increased and S. simulans has decreased in prevalence. The predominant CNS isolated from all three herds were S. chromogenes (48%), S. hyicus (26%), and S. epidermidis (10%). While these herds have similar CNS present, no common pulsotype was seen among the three farms evaluated. Results from this study also suggest that CNS can persist in the mammary gland for at least 10 months and that mammary quarters infected by CNS can contribute significantly to the number of somatic cells in milk.

References
P004 Internalization by mammary epithelial cells of coagulase-negative staphylococci isolated from bovine mastitis

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Introduction: Coagulase-negative staphylococci (CNS) have become very common as mastitis causing agents. In many countries they have become the predominant pathogen (Pitkälä et al., 2004; Tenhagen et al., 2006). Although not as pathogenic as the so-called major pathogens, CNS can cause persistent infections (Aarestrup et al., 1999; Taponen et al., unpublished). Possible internalization of the persistent and non-persistent isolates of CNS has not been studied. In the present study, we investigated the adhesion, invasion and multiplication of CNS isolated from clinical mastitis using a bovine mammary epithelial (BME) cell model.

Materials and methods: CNS isolates (19) from bovine mastitis (8 Staphylococcus chromogenes, 4 S. simulans, 3 S. epidermidis, 2 S. haemolyticus and 2 S. cohnii strains) were identified as described before (Taponen et al., 2006). Seven of the nineteen CNS strains were from persistent and five from transient infections (Taponen et al. unpublished data). A non-pathogenic S. epidermidis strain S808 isolated from cheese was used as a negative control and a reference strain S. aureus ATCC 2592 and S. aureus 298 isolated from clinical mastitis were used as positive controls. BME cells (BME-UV1, provided by prof. C. Burvenich, Ghent, Belgium) were grown in 24-well cell culture plates at 37°C in 5% CO₂ atmosphere using Ham’s F12 medium (40%) (23% RPMI 1640, 20% NCTC 135, 10% FBS, 1% lactose, 1% lactalbumin hydrolysate, 2% antibiotic-antimycotic solution, 2% hydrocortisone, 0.5% L-ascorbic acid, 0.03% mM GSH and 0.01% insulin). Confluent monolayers of BME cells of the fifth to seventh passage were subcultured once in antibiotic free medium before bacterial exposure. Adhesion, invasion and multiplication were determined as described by Almeida and Oliver (2001) with modifications. Co-cultures were incubated for 0.5, 2 and 21 h. The CFUs of adherent, invaded and internalized bacteria were determined by plating and counting after overnight incubation at 37°C.

Results: In co-cultures of BME cell monolayers and control strains, S. aureus ATCC 2592, S. aureus 298 and the non-pathogenic control S. epidermidis S808 adhered similarly, but S. aureus strains invaded better than S. epidermidis. The multiplication rate of S. aureus isolated from clinical mastitis was higher than the observed with S. aureus ATCC 2592 and with the non-pathogenic S. epidermidis of food origin. The intensity of adhesion and invasion among CNS strains was weaker than in S. aureus strains. The replication of CNS strains was lower than that of S. aureus mastitis strain, but 13 out of 19 CNS strains had a higher ratio than the reference strain S. aureus ATCC 2592. Adhesion and invasion as well as multiplication and multiplication ratio had a strong positive correlation during the exposure (Table). No differences were found between the persistent and transient CNS strains in adhesion, invasion or multiplication ratio (Figure).

Discussion and conclusions: The adhesive and invasive capacity of bacteria are important virulence characteristics. Many pathogens causing intramammary infection are capable of adhering to and invading mammary epithelial cells (Mathews et al., 1994; Almeida and Oliver, 1995 and 2001; Almeida et al., 1996; Dogan et al., 2006). Bacterial adherence seems to be essential as the first stage for internalization of these pathogens into the host cells. CNS are often considered less pathogenic than the major pathogen S. aureus. Results from this study show that the investigated CNS species, S. chromogenes, S. simulans, S. epidermidis, S. haemolyticus and S. cohnii, have an equal adhesive ability as S. aureus,
but internalization varies between them. According to Dogan et al. (2006) transient and persistent Escherichia coli strains both adhered, but the persistent strains invaded more efficiently than the transient strains. No difference in the ability to adhere to, invade and replicate in cultured mammary epithelial cells was found between persistent and transient CNS strains. The number of CNS strains investigated was small, so these results should be considered as indicative only.

References:

Table. Correlation between adhesion, invasion and multiplication during the bacterial exposure

<table>
<thead>
<tr>
<th></th>
<th>Adhesion</th>
<th>Invasion</th>
<th>Multiplication</th>
<th>Multiplication rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion</td>
<td>1</td>
<td>0.779</td>
<td>0.594</td>
<td>0.213</td>
</tr>
<tr>
<td>Invasion</td>
<td>0.779</td>
<td>1</td>
<td>0.627</td>
<td>0.186</td>
</tr>
<tr>
<td>Multiplication</td>
<td>0.594</td>
<td>0.627</td>
<td>1</td>
<td>0.763</td>
</tr>
<tr>
<td>Multiplication rate</td>
<td>0.213</td>
<td>0.186</td>
<td>0.763</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure. Multiplication rate and invasion % of the persistent (●), transient (▲) CNS strains and the positive control strains (◊), S. aureus ATCC 2592 and S. aureus 298. The negative control (□) S. epidermidis S808 is also shown.
Coagulase negative staphylococcal mastitis in dairy farms of Latvia

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Introduction: Udder inflammation is one of widespread cows’ diseases. Mastitis in Latvia equally with other countries, where dairy farming is well developed, is in the first place among diseases. It is approved in all herds and almost half of all dairy cows are diseased with mastitis. With clinical mastitis 6% of cows, but with subclinical mastitis up to 30 and more per cent of all dairy cows are diseased. Also in Latvia, as in other countries, replacement of predominating agents of mastitis from the genus Streptococcus to the genus Staphylococcus has been observed. The aim of present study was to clear up species of coagulase negative staphylococci (CNS) involved in clinical and subclinical mastitis of lactating cows.

Material and Methods: Udder secretion samples (n = 398) from 398 cows in different dairy farms in the Riga region were collected. The cows were of two different breeds – Latvia’s Brown and Black and White. Udder secretion samples were collected from 311 cows with subclinical mastitis, and from 87 cows with clinical mastitis. The majority of cows selected for this study were in the second to fourth lactation. The presence of bacteria in the udder secretion samples was determined at the Laboratory of the Department of Veterinary Medicine in the Research Institute “Sigra”. For bacteriological examination, 0.05 ml aliquot of each sample was inoculated on different complex and selective culture media. Colonies of CNS were further identified using Gram – positive kits of BBL Crystal Identification System.

Results: Gram-positive cocci from the genera Staphylococcus, Streptococcus and Aerococcus were isolated from clinically and subclinically diseased cows’ udder secretion samples. From gram-positive cocci, the more frequently isolated bacteria were from the genus Staphylococcus both in clinically and subclinically diseased cows’ udder secretion samples – in 229 (74.1 %) and 41 (48.2 %) cases, respectively (Table 1). Among the species of genus Staphylococcus (S.), S. aureus predominated in subclinically, but CNS and coagulase positive S. intermedius in clinically diseased cows’ udder secretion samples. Coagulase negative S. haemolyticus, S. simulans, S. xylosus, S. capitis, S. saprophyticus, S. epidermidis, and S. vitulus were established in subclinically diseased, but S. haemolyticus and S. kloosii in clinically diseased cows’ udder secretion samples (Table 2). S. haemolyticus was isolated both in clinical and subclinical mastitis cases more frequently. Coagulase positive S. intermedius, which was isolated in 6 (7.7 %) cases from subclinically and in 6 (26.1 %) cases from clinically diseased cows’ udder secretion samples, is a considerable pathogen, which causes not only mastitis but also various other infections.

Conclusions: Microorganisms of the genus Staphylococcus predominate in subclinically as well as in clinically diseased cows’ udder secretion samples. Seven CNS species were isolated from the subclinically, but two from clinically diseased cows’ udder secretion samples. S. haemolyticus is the most frequently isolated CNS species from the diseased cows’ udder secretion.

References:


Table 1: The microorganisms isolated from subclinically and clinically diseased cows’ udder secretion samples

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>Subclinical mastitis</th>
<th>Clinical mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>229</td>
<td>74.1</td>
</tr>
<tr>
<td>Aerococcus spp. and Micrococcus spp. associations</td>
<td>35</td>
<td>11.3</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>25</td>
<td>8.1</td>
</tr>
<tr>
<td>Aerococcus spp.</td>
<td>7</td>
<td>2.2</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Fungi</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non isolated</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>311</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2: The Staphylococcus species isolated from clinically and subclinically diseased cows’ udder secretion samples

<table>
<thead>
<tr>
<th>Isolated staphylococci species</th>
<th>Subclinical mastitis</th>
<th>Clinical mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>37</td>
<td>47.4</td>
</tr>
<tr>
<td>S. simulans</td>
<td>8</td>
<td>10.3</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>8</td>
<td>10.3</td>
</tr>
<tr>
<td>S. capitis</td>
<td>8</td>
<td>10.3</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>6</td>
<td>7.7</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>S. vitulus</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>S. kloosii</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Introduction: Bovine mastitis is a frequent cause of economic loss in dairy herds. The epidemiology of bovine intramammary infections (IMIs) has been characterized worldwide by an increase in the prevalence of Staphylococci. The staphylococci are the predominant pathogen in subclinical and chronic bovine mastitis. Staphylococci are present as major mastitis pathogens in the dairy industry worldwide. Coagulase-negative staphylococci (CNS) are increasing in importance as causes of bovine IMI throughout the world in recent years. CNS have been isolated from milk samples collected from cows with clinical and subclinical mastitis in several countries, cause tissue damage and decreases in milk production. The purpose of this study was to determine the prevalence and in vitro antibiotic susceptibility of CNS isolated from bovine IMI in Argentina to several antimicrobial agents used in the control of this disease.

Materials and Methods: Milk samples were taken aseptically from all quarters of 300 bovine infected udders of some dairy industry farms of Mashhad, Iran. 0.01 ml of each sample was cultured to determine the presence of CNS. A quarter was identified as infected when a single pathogenic bacterium was isolated and SCC were increased above 200,000/ml. The milk samples were plated out on blood agar plates with 5% defibrinated sheep blood and incubated at 37°C for 24-48 h. After incubation, suspected colonies were stained by Gram staining technique and were examined using light microscope. Gram positive cocci were further identified by biochemical tests. The antibiotic susceptibility tests for CNS isolates from mastitis milk samples were carried out by disk diffusion as described by Kirby-Bauer using disks of penicillin, 10 IU; oxacillin, 1 µg; ampicillin, 10 µg; neomycin, 30µg; kanamycin, 30µg; gentamicin, 10 µg; erythromycin, 15 µg; tetracycline, 30µg; enrofloxacine, 5µg; and cephalotin, 30µg. Isolates were categorized as susceptible, intermediate, and resistant based upon interpretive criteria developed by the National Committee of Clinical Laboratory Standards. Staph. aureus ATCC 25923 was used as a control in all of the assays.

Results: From a total of 300 milk samples collected, 67 (22.33%) were positive for coagulase negative staphylococci. CNS strains were identified as 18 (26.86%) strains of Staph. hyicus, 14 (20.89%) strains of Staph. chromogenes, 15 (22.39%) strains of Staph. epidermidis, 10 (14.92%) strains of Staph. haemolyticus, 3 (4.48%) strains of Staph. sciuri, 4 (5.97%) strains of Staph. simulans and 3 (4.48%) strains of Staph. xylosus. The antibiotic susceptibility test results showed that all CNS strains were susceptible to gentamycin and cephalotin and resistant to penicillin (100%) and oxacillin (78%).

Conclusion: Results of this study showed that coagulase negative staphylococcus is emerging as important minor pathogens and can be the cause of substantial economic losses. The high resistance to penicillin found in this study emphasizes the importance of the identification of coagulase negative staphylococcus when mastitis is present.

References:
P007 Antimicrobial resistance and strain persistence in coagulase-negative staphylococci over the dry period

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Introduction: The importance of coagulase-negative staphylococci (CNS) has recently been reviewed. They are the most commonly isolated organisms in bovine intramammary infections (IMI) and their prevalence is generally highest at calving in all parities. This is despite the fact that in many countries all cows are routinely treated with antimicrobial drugs at the end of lactation and that the cure rates of CNS over the dry period are high. Numerous CNS species have been identified and isolated from cases of bovine mastitis and may differ in their virulence and ability to persist.

Emerging antimicrobial resistance (AR) in human and veterinary medicine has become a worldwide concern and staphylococcal species have been shown to be associated with AR related problems and zoonoses. The objective of this study was to elucidate the persistence and antimicrobial resistance of CNS with respect to antimicrobial dry cow therapy.

Materials and methods: Data for this project came from an ongoing study evaluating the effect of selective dry cow therapy on udder health in US dairy herds. Based on monthly composite SCC at the end of lactation and clinical mastitis history during the lactation, cows in four Ohio dairy herds were assumed either as infected or uninfected. All “infected” cows received antibiotic dry cow treatment while “uninfected” cows were randomly assigned to receive or not to receive antibiotic dry cow treatment. Quarter milk samples were collected for microbiological culture at dry-off and within 3 days of calving. Isolates were frozen and stored at -80°C for further analysis. CNS isolates were speciated using API Staph biochemical test kits according to manufacturer’s guidelines (BioMerieux). Genotypic characterization of the isolates was performed using pulsed field gel electrophoresis (PFGE). Antimicrobial susceptibility of the isolates was determined using Sensititer System (Trek Diagnostic, Westlake, OH) and a commercially available broth microdilution panel designed for mastitis pathogens. The panel included 10 antimicrobials used in mastitis treatments: penicillin (pen), ampicillin (amp), oxacillin (oxa), cephalothin (cep), ceftiofur (cft), penicillin+novobiocin (pnov), erythromycin (ery), pirlimycin (pir), tetracycline (tet), and sulfadimethoxine (sd). Multi-drug resistance was defined as resistance to 3 or more antimicrobials.

Results: To date, over 800 cows have been sampled at dry-off and calving. Coagulase-negative staphylococci were the most commonly isolated organisms at both time points regardless of the treatment status of the cows. Of all quarters sampled, 24.2% and 40.8% were positive for growth at dry-off and at calving, respectively. Of those isolates, 63.6% and 61.7% were CNS at dry-off and at calving, respectively. Antimicrobial susceptibility testing has been performed on 468 CNS isolates. Of the tested isolates, 34.8% were pansusceptible, with no difference between dry-off and calving. Resistance levels to all tested antimicrobials were higher at calving than at dry-off, however, not all differences were statistically significant. Resistance was also higher for organisms isolated from cows that were treated at dry-off when compared to those not treated. Overall, 25.7% of the isolates (31.3% and 18.7% of isolates from treated and untreated cows, respectively) were multiresistant.

To date, genotypic characterization has been performed on selected isolates from several cows within a single herd. Isolates collected at both dry-off and calving and from multiple quarters within individual cows with similar and/or different phenotypic resistance patterns were characterized. Preliminary results from the PFGE data are shown in Figure 1.
Conclusions: Preliminary results suggest that antimicrobial resistance levels may increase over the dry period in populations of CNS isolates. Preliminary PFGE data have some interesting implications. First, the clonality of strains isolated from cows at dry-off and at calving indicated persistence even after treatment. Second, genotypic diversity within a cow as well as clonal strains with different phenotypic resistance patterns were found. Further studies on clonality, diversity and genetic resistance components will help elucidate the role of dry cow therapy on strain persistence and antimicrobial resistance in CNS.

References
5. Taponen, S. and S. Pyörälä. 2007. How important is coagulase-negative Staph as a cause of mastitis? 46th Annual Meeting of NMC. San Antonio, TX, USA.

![PFGE genotyping of 17 CNS isolates from five cows in one herd at different sampling times (dry-off, D and calving, C) from different quarters (LF, LR, RF and RR). All isolates except for 794-2352 were from treated cows. At 95% genetic similarity threshold (marked by the dashed line), five clusters and three individual clones were indicated.](image-url)
Somatic cell counts and severity of subclinical mastitis caused by coagulase-negative staphylococci in heifers

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Introduction: Somatic cell count (SCC) is a reflection of the inflammatory response to an intramammary infection. There is general agreement between the infection status and the inflammatory response to this infection (7). Coagulase-negative staphylococci (CoNS) are found among the normal flora of the skin and mucous membranes, they long have been regarded as skin commensals and as culture contaminants. In recent years, their important role as pathogens and their increasing incidence have been recognized. CoNS, among them Staphylococcus chromogenes, were isolated frequently from unbred and primigravid heifers (1, 8, 5, 6) and caused the majority of prepartum intramammary infections (6, 2). In heifers in their first lactation, among the CoNS, S. intermedius, S. chromogenes and S. haemolyticus were the most frequently isolated. Milk from CoNS-infected quarters had significantly higher SCC than milk from infection-free quarters (3). The objective of this study was to ascertain the severity of subclinical mastitis caused by CoNS using a comparison of SCC values accompanying the mammary gland infections with other mammary pathogens.

Materials and Methods: The study was performed in group of 51 virgin heifers (Holstein and Czech Spotted cross-breeds) in the terms: i) before breeding and ii) after the first delivery in the same animals. Mammary gland lavages, volume standardised, were taken in the virgin heifers by the procedure described previously (6). In heifers after delivery, repeated, twofold, quarter foremilk samples were obtained. Mastitis pathogens were detected by the procedure described in the hand book of the National Mastitis Council (4). CoNS were detected by the inoculation of 0.1 ml milk sample smears on Baird-Parker agar. After 48 hours of incubation at 37°C, the typical colonies were subcultured on blood agar and incubated for 24 hours at 37°C. A Catalase test and tube coagulase test were conducted. The API Staph system was used for species-level identification. Somatic cell counts were detected using the Fossomatic 90 apparatus. Logarithmically transformed data underwent statistical analysis by Student’s t-test (data related to virgin heifers) and ANOVA with Schéffe’s test (data related to heifers after first calving).

Results: The study has documented that CoNS caused the majority of intramammary infections in virgin heifers (10.8% out of 204 udder quarters; 8.8% caused other pathogens; 80.4% quarters were free of infection). A statistically highly significant higher value of SCC was found in contrast to pathogen-free samples (log meanfree = 1.327/ml; log meanCoNS = 2.063/ml; P≤0.01). Infection with other mammary pathogens was accompanied with gradually increase values of SCC (environmental pathogens log mean = 5.792/ml; S. aureus log mean = 6.486/ml). In heifers after the first calving, the values of log means SCC were increased in the order: i) pathogen-free = 1.327/ml; ii) CoNS = 2.063/ml; iii) environmental pathogens = 2.465/ml; iv) S. aureus infections = 2.585/ml. The differences were statistically highly significant between i) and all the other pathogens (P≤0.01). The difference between ii) and iii) was only statistically significant (P≤0.05). The difference between iii) and iv) was not statistically significant (P≥0.05) (see Table 1). In heifers after first calving, the proportions of infected udder quarters grouped by causative pathogenic agents were: CoNS = 11.3%; environmental pathogens = 9.3%; S. aureus infections = 11.8%; quarter free of infection = 67.6%.

Conclusions: In conclusion it was stated that mammary infection caused by CoNS presents only a mild severity inflammatory process in comparison with other mammary pathogens in
Heifers. CoNS caused the majority of intramammary infections in the virgin heifers. In heifers after the first calving, the proportions of CoNS and S. aureus infected quarters were approximately equal.

References:

The study was drawn up with support by the Ministry of Agriculture of the Czech Republic (MZE 0002716201).

Table I. The significance of differences in milk somatic cell counts between pathogens-free and infected udder quarters in heifers after the first calving ANOVA and Schefte’s test.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Somatic Cell Counts [log mean/ml]</th>
<th>Somatic Cell Counts * [mean x 10^3/ml]</th>
<th>Differences [log mean/ml]</th>
<th>t-values</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>1.3270</td>
<td>21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i : ii</td>
<td>2.0634</td>
<td>115.7</td>
<td>0.7363</td>
<td>7.7281</td>
<td>P≤0.01</td>
</tr>
<tr>
<td>i : iii</td>
<td>2.4654</td>
<td>294.0</td>
<td>1.1383</td>
<td>10.9935</td>
<td>P≤0.01</td>
</tr>
<tr>
<td>i : iv</td>
<td>2.5853</td>
<td>384.8</td>
<td>1.2583</td>
<td>13.4492</td>
<td>P≤0.01</td>
</tr>
<tr>
<td>ii : iii</td>
<td>2.4654</td>
<td>294.0</td>
<td>0.4020</td>
<td>3.0604</td>
<td>P≤0.05</td>
</tr>
<tr>
<td>ii : iv</td>
<td>2.5853</td>
<td>384.8</td>
<td>0.5220</td>
<td>4.2218</td>
<td>P≤0.01</td>
</tr>
<tr>
<td>iii : iv</td>
<td>2.5853</td>
<td>384.8</td>
<td>0.1200</td>
<td>0.9220</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

i - pathogen-free; ii - coagulase-negative staphylococci; iii - environmental pathogens; iv - S. aureus
* delogarithmed mean value (i.e. geometric mean)
N.S. none significance
Introduction

Bovine mastitis is the leading cause of economic losses in dairy herds worldwide. Diverse organisms like bacteria, mycoplasma, yeasts, and algae have been attributed as etiological agents for mastitis. In the last four decades, there has been a drastic change in the prevalence of mastitis-causing pathogens. Currently, environmental mastitis is the major problem on modern, well-managed dairy farms. Coagulase-negative Staphylococcus species (CNS) are among the most commonly isolated bacterial species from the bovine mammary gland. The CNS group is a collection of more than 30 species and subspecies. The role of CNS in bovine mastitis has come under increased scrutiny with more isolates associated with clinical and subclinical intramammary infections (2, 4).

Antimicrobial agents are used commonly to control and/or to prevent bacterial infections in lactating and nonlactating cows and heifers. Thus, in vitro susceptibility testing is used as a tool to guide the veterinarian in selecting the most efficacious antimicrobial agents to use when treating cows. Several reports on CNS including antimicrobial susceptibility data have considered CNS as one homogenous group. However, more recent studies have shown species-specific differences in the ability of CNS to persist in the udder suggesting that some CNS may be more problematic in the mastitis syndrome. In the present study, we investigated CNS isolated in milk from infected cows and heifers for their susceptibility to antimicrobials used commonly for mastitis therapy.

Material and Methods

A total of 168 CNS were isolated from quarter milk samples from three dairy research farms of The University of Tennessee. Bacteria were isolated originally from cows with clinical or subclinical mastitis and stored at -80°C following identification. Revived isolates were confirmed using API 20 Staph (bioMerieux Inc., Hazelwood, MO, USA). All isolates were sub-cultured on blood agar at 35°C for 24 h prior to antimicrobial susceptibility testing.

In vitro antimicrobial susceptibility testing was conducted using a custom prepared micro-dilution panel (Trek Diagnostics, Cleveland, OH, USA). All minimum inhibitory concentration (MIC) determinations were performed according to methods described by the Clinical and Laboratory Standards Institute (1) document M7-A6. Briefly, broth dilutions were made using cation-adjusted Mueller-Hinton broth (Becton Dickinson, Sparks, MD, USA). The inoculum was prepared by growing cultures to log phase and standardized to achieve a final concentration of approximately 5 x 10⁴ colony forming units/well. Plates were incubated at 35°C for 20-24 h before reading MICs. MIC end points were determined according to CLSI document M7-A6. Reference strains from the American Type Culture Collection (ATCC), Manassas, VA, USA including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, and Enterococcus faecalis ATCC 29212 were used.

Genetic relatedness/clonal similarity of S. epidermidis was determined by pulsed-field gel electrophoresis (PFGE) analysis of genomic DNA digested with restriction enzymes. PFGE fingerprints were determined and compared following procedures for Gram-positive bacteria described by McDougal et al. (3). Gel DNA band patterns were analyzed using Molecular Analyst software version 1.6 (Bio-Rad Laboratories, Hercules, CA, USA) to determine strain relatedness. The Jaccard binary coefficient, which considers presence or absence of bands,
was used to construct dendrograms and to determine strain similarities. Band position tolerance of 2.5% was used for comparison of DNA patterns.

Results
A total of 168 isolates were evaluated for MIC to antimicrobials used commonly for mastitis therapy. The predominant species evaluated were S. chromogenes (n=61), S. epidermidis (n=37), S. hyicus (n=37), and S. simulans (n=16). Most CNS exhibited high susceptibility to β-lactams such as ampicillin, oxacillin, cephalothin, and ceftiofur. Susceptibility to combination antimicrobials such as lincomycin/neomycin and penicillin/novobiocin was observed. In addition, erythromycin and pirlimycin were very effective in vitro inhibitors of CNS.

The only exception was observed with S. epidermidis. Of the 37 S. epidermidis evaluated, 13 (35%) exhibited resistance to erythromycin (≥16 µg/ml) and one was resistant to both erythromycin (≥64 µg/ml) and pirlimycin (≥64 µg/ml). A total of 17 S. epidermidis, 10 S. chromogenes, and 1 S. hyicus exhibited phenotypic resistance to ampicillin (≥0.5 µg/ml). Resistance to oxacillin (≥0.5 µg/ml) was predominantly observed in S. epidermidis (n=12; 32%). All isolates that were resistant to oxacillin were also resistant to ampicillin and erythromycin. Multidrug resistant S. epidermidis (n=12) isolates were distributed among four closely related pulsotypes (≥86% similarity index) out of 22 total pulsotypes. Interestingly, all multidrug resistant strains except one were from the same farm. The same multidrug resistant pulsotypes were isolated from different cows (≥2 to 5). This wide dissemination was not observed in antimicrobial susceptible S. epidermidis isolates.

Conclusions
Among the CNS that were evaluated in the present study, S. epidermidis appears most likely to be a reservoir of antimicrobial resistance determinants. PFGE data suggests that multidrug resistant S. epidermidis may have the ability to persist for prolonged periods of times and disseminate among the herd population. These isolates are currently being evaluated for additional virulence determinants. Only one S. epidermidis isolate was resistant to erythromycin and pirlimycin but was genotypically dissimilar from multidrug resistant isolates. Methicillin resistance (oxacillin) in S. epidermidis should be of concern as it implies resistance to all beta-lactams. Results from this and other studies suggest that CNS isolated from bovine udders should not be dealt with as a homogenous group of mastitis pathogens and their designation as minor mastitis pathogens should be questioned.

References


P010 Pathogenicity factors and antimicrobial resistance of Staphylococcus epidermidis isolated from bovine mammary glands

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Introduction
Bovine mastitis is the leading cause of economic losses in dairy herds throughout the world. Diverse organisms like bacteria, mycoplasma, yeasts, and algae have been attributed as etiological agents for mastitis. Coagulase-negative Staphylococcus species (CNS) are among the most commonly isolated bacterial species from the bovine mammary gland. Historically, virulence of mastitis pathogens has been determined by the magnitude of the specific cellular response. The somatic cell count (SCC) in milk from mammary glands subclinically infected by CNS is typically 250 x 10^3/mL or less, which was previously considered normal. This lead to the classification of CNS as minor pathogens. In current scenarios where the SCC of milk from uninfected mammary glands is known to be much lower (~50 X 10^3), CNS have been revisited and their classification as minor mammary gland pathogens is being reconsidered. Staphylococcus epidermidis is a major cause of nosocomial infections and frequently associated with catheter and medical device related sepsis in human medicine. In the last years, Staph. epidermidis has emerged as an important mastitis pathogen. There has been much interest currently to identify virulence factors associated with Staph. epidermidis infections. It has been reported previously that persistence of staphylococcal infections in the udder is associated with various virulence factors such as production of exotoxins and surface proteins. The formation of highly organized multicellular complexes by bacteria, known as biofilms is increasingly being recognized as an important virulence factor in Staph. epidermidis (3). Pathogenicity of Staph. epidermidis has also been linked to resistance to antimicrobial agents. Objectives of the present study were to evaluate antimicrobial susceptibility and pathogenicity of Staph. epidermidis isolated in milk obtained from heifers and cows with clinical or subclinical mastitis.

Material and Methods
A total of 37 Staph. epidermidis were isolated from quarter milk samples of heifers and cows with clinical or subclinical mastitis from three dairy research farms of The University of Tennessee. Bacteria were stored at -80°C following identification. Revived isolates were confirmed using API 20 Staph (bioMerieux Inc., Hazelwood, MO, USA). All isolates were sub-cultured on blood agar at 35°C for 24 h prior to antimicrobial susceptibility testing. In vitro antimicrobial susceptibility testing was conducted using a custom prepared micro-dilution panel (Trek Diagnostics, Cleveland, OH, USA). All minimum inhibitory concentration (MIC) determinations were performed according to methods described by the Clinical and Laboratory Standards Institute (1) document M7-A6. Briefly, broth dilutions were made using cation-adjusted Mueller-Hinton broth (Becton Dickinson, Sparks, MD, USA). The inoculum was prepared by growing cultures to log phase and standardized to achieve a final concentration of approximately 5 x 10^4 colony forming units/well. Plates were incubated at 35°C for 20 - 24 h before reading MICs. MIC end points were determined according to CLSI document M7-A6. Reference strains from the American Type Culture Collection (ATCC), Manassas, VA, USA including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, and Enterococcus faecalis ATCC 29212 were used. Isolates were tested for β-lactamase production by the nitrocefin test (cefinase discs, Becton Dickinson Microbiology Systems, Sparks, MD, USA). Cefinase disks impregnated with nitrocefin, a chromogenic cephalosporin that exhibits red color change from yellow to red as
the β-lactamase ring is hydrolyzed, were used. Staphylococcus aureus ATCC 29213 was used as a positive control. All negative isolates were induced with 1 µg oxacillin disk and retested for β-lactamase activity.

Whole cell DNA was prepared using DNeasy® Tissue Kit (Qiagen Inc., Valencia, CA, USA). PCR assays for ribosomal methylase genes erm(A), erm(B), erm(C) and ATP-binding transporters msrA were performed in isolates that exhibited elevated MICs to pirlimycin and/or lincomycin, and erythromycin. Staphylococcus epidermidis isolates with an elevated MIC to oxacillin were evaluated for the presence of mecA. All isolates that exhibited nitroceftin activity were evaluated for the presence of blaZ. All Staph. epidermidis were also evaluated for the virulence factor incAB. PCR was performed using PuReTaq™ Ready To-Go PCR beads (GE Healthcare, Buckinghamshire, UK). A sample of each PCR amplified product was purified and sequenced at the Molecular Biology Core Sequencing Facility, The University of Tennessee, Knoxville, TN, USA.

Results
Of the 37 Staph. epidermidis evaluated, 13 (35%) exhibited resistance to erythromycin (≥16 µg/ml) and one was resistant to both erythromycin (≥64 µg/ml) and pirlimycin (≥64 µg/ml). The 13 Staph. epidermidis isolates contained msrA, whereas Staph. epidermidis isolates resistant to erythromycin and pirlimycin carried ermC. Based on the nitroceftin test, resistance to ampicillin was attributed to constitutive (n=14) and induced (n=23) β-lactamase production. On the MIC panel, only isolates that exhibited constitutive β-lactamase production exhibited resistance to ampicillin (≥0.5 µg/ml). Regardless of the type of β-lactamase expression, most Staph. epidermidis (all but two) carried blaZ that encodes for β-lactamase. Staphylococcus epidermidis isolates that exhibited resistance to oxacillin (≥0.5 µg/ml) carried mecA. Resistance to macrolides and β-lactams including methicillin (oxacillin) was more common in constitutive β-lactamase producers. These Staph. epidermidis isolates also carried incAB virulence gene cluster that encodes for polysaccharide intercellular adhesion.

Conclusions
Penicillin resistance encoded by blaZ cannot always be detected by MIC susceptibility assays in Staph. epidermidis. A subgroup of isolates possessed multidrug resistance and virulence factors that have been commonly observed in hospital-associated invasive Staph. epidermidis strains (2). The mecA found in Staph. epidermidis isolated from dairy cows with mastitis shared close sequence homology to mecA from community-acquired methicillin resistant Staphylococcus aureus. The incAB is involved with biofilm production and may play a role in enhanced antimicrobial resistance as well as persistent and chronic infections of the bovine mammary gland.

References
Comparison of tDNA-intergenic spacer PCR and rpoB-gene sequencing for species-level identification of coagulase-negative staphylococci

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Introduction: In many European dairy farms that have adopted the 5- and 10-point mastitis prevention programs, the relative importance of coagulase-negative staphylococci (CNS) has increased. They are the predominant pathogens found in milk samples and are causing the majority of intramammary infections (imi) in fresh dairy heifers. The increase in CNS prevalence and incidence relative to traditional major pathogens, combined with changes in limits for bulk milk somatic cell count penalties and the fact that CNS are causing clinical mastitis also, warrant reconsideration of their historical designation "minor pathogen". On the other hand, protective characteristics of CNS have been reported. The confusion can partly be explained by the lack of (accurate) species identification. Accurate and low-cost identification is a prerequisite for epidemiological studies aiming at elucidating the relevance of the different CNS species in bovine mastitis. Current identification methods are largely phenotypic and based on reference strains of human origin. These methods may not be suitable for isolates of bovine origin. We compared tDNA-intergenic spacer PCR with sequencing of the rpoB-housekeeping gene for identification of bovine CNS species.

Materials and methods: Through the Dutch Animal Health Service lab, 92 CNS isolates were available from milk samples from cows with clinical and subclinical mastitis. DNA-lysates were prepared by alkaline extraction1. tDNA-intergenic spacer PCR was performed as described1,2,4. The length of the PCR-products was analysed with capillary electrophoresis using an ABI-Prism™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)1,2,4 and a software program was used for interpretation1. Sequencing of the rpoB-gene was performed as described3 with small modifications. PCR-products were purified with PureLink DNA purification kit (Invitrogen) and sequencing was performed at the BioResource Center at Cornell University using the PCR-primers. Results were analysed with the DNA-star programs (Lasergene) and compared to reference data using nucleotide-nucleotide BLAST.

Results: Sequencing of the rpoB-gene resulted in a reliable identification (>98% agreement with one of the available reference strains) for 78 isolates. S. chromogenes was the most prevalent species (39%). S. warneri (10 isolates) was identified with a certainty of 97% but identification was considered correct because of the large difference with the next best match (89%). A cluster of 4 S. hyicus isolates that had only a 94% match with reference strains was identified. Additional 16S- and cnp60-sequencing will be performed. For 6 (6.5%) and 23 isolates (25%) no identification was obtained with sequencing and tDNA-PCR, respectively. Currently, 71% of the identifications done with the tDNA-PCR were in agreement with the rpoB-sequencing results (Table 1). In 3 cases, tDNA-PCR failed to differentiate between S. hyicus and S. chromogenes. Disagreement was seen for S. fleuretti and S. arlettiae.

Discussion: When studying the impact of different CNS species on udder health in dairy cattle, an accurate identification technique is required. Although no single test can offer fully

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reliable identification of bacterial species, gene sequencing is often seen as the gold standard. Unfortunately, the high cost limits its use in large field studies for most routine laboratories. Phenotypic methods on the other hand are usually cheaper but lack accuracy. The results of this study show that tDNA-intergenic spacer PCR could be a good alternative for gene sequencing. It’s a rapid, low-cost and easy to perform technique that has a high reproducibility if capillary electrophoresis is available. tDNA-PCR had some difficulties in differentiating S. chromogenes and S. hyicus. For one sequence, found in 4 isolates, rpoB-sequencing showed 94% identity to S. hyicus, which is not considered sufficient; additional sequencing of the 16S- and cpn60-genes might give a definite answer (in progress). For tDNA-PCR as well as for gene sequencing, availability of a reference database is a prerequisite. Currently, the reference database for sequence data is larger than the one for tDNA-PCR. Updating the current database used for tDNA-PCR identification could improve its accuracy (in progress). To conclude, tDNA-PCR could be a useful tool for large field studies aiming at elucidating the relevance of CNS imi in dairy cattle.

References:

Table 1: Identification of CNS isolates based on sequencing of the rpoB-gene and on tDNA-intergenic spacer PCR.

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<td>S. haemolyticus</td>
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<tr>
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P012 Effects of intramammary injections of rbGM-CSF and rbIL-8 on milk CL activity, SCC and counts of total bacteria and staphylococci in Holstein cows with staphylococcal mastitis.

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Introduction: Polymorphonuclear leukocytes (PMN) play an important role in the host defences of cattle against pathogens. For example, infection of the mammary glands by bacterial pathogens is characterized by a rapid influx of PMN into the infected glands where they are responsible for phagocytizing and killing the microbes. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-8 (IL-8), which are members of the chemokine family of cytokines promote the bactericidal activity and the chemotaxis of PMN and macrophages. In previous studies, the authors demonstrated that recombinant bovine GM-CSF (rbGM-CSF)2 and rbIL-83 had potentials as therapeutic agents of the subclinical mastitis of dairy cows. The present study investigated that the effects of combined intramammary injections of the rbGM-CSF and rbIL-8 on quarter milk levels of chemiluminescence (CL) activity1 and somatic cell count (SCC) and shedding patterns of total bacteria and staphylococci in an udder suffering from naturally infected subclinical mastitis.

Materials and Methods: Fifteen Holstein dairy cows were used. They were all in mid-lactation and were milked twice daily throughout the study. These animals were selected through periodical milk screenings of a herd of lactating cows and were divided into an early-stage group (7 cows) and a late-stage group (8 cows). The former group was within 1 month, and the latter group was between 2 and 6 months after the spontaneous infection of staphylococci. The rbGM-CSF was produced by Baculovirus expression system2. The rbIL-8 was produced by Brevibacillus choshinensis expression system3. The mixture of saline and the bacterial infected serum-free culture fluid (9 : 1 v/v) was used as a control solution. A sterile polyethylene catheter (outer diameter; 1 1/3 mm) was inserted into the cistern of a staphylococcal subclinical mastitis-suffered quarter through the teat canal. As a control experiment, 5 ml of control solution was injected within 30 s into the cistern through the catheter. The injection was conducted twice, namely, just after the morning and evening milking. Seven days after the injections of the control solution, 0.4 mg/5ml saline of rbGM-CSF2 and 1 mg/5 ml saline of rbIL-83 were injected within 30 s into the same cistern just after the morning and the evening milking, respectively.

Results: None of the cows in the early- and late-stage groups showed any abnormal clinical symptoms or any visible local reactions in the areas injected with control or cytokine solutions. In both the groups, rectal temperature had a transient rise (approximately 0.4 ) 6 h after the injections of control or cytokine solutions. The daily milk yield was little affected by the injections of control or cytokine solutions in both the groups. In the early-stage group, the milk CL activity and SCC had significant increases day 1, and were followed by smooth declines to under pre-injection values on day 14. In the late-stage group, the values had also significant increases day 1. However, the milk components maintained high levels thereafter following the cytokine injections (Figure 1(a), (b)). Counts in milk total bacteria and staphylococci were markedly lowered at 6 h and were maintained low levels thereafter following the cytokine injections in the early-stage group. In the late-stage group, the counts were also lowered at 6 h and day 1 by the cytokine injections. However, the bacterial counts rose back on days 7 and 14 of cytokine injections (Figure 1(c), (d)).
**Conclusion:** Combination injections of rbGM-CSF and rbIL-8 cause the therapeutic effect on the staphylococcal mastitis of dairy cows, if the cytokines are applied at an initial stage of infection.

**References:**

**Figure 1:** Changes in milk chemiluminescence activity (a), somatic cell count (b) and counts of total bacteria (c) and staphylococci (d) following intramammary injections of control (dotted line), rbGM-CSF and rbIL-8 solutions (solid line) in early-stage ( ; n=7) and late-stage ( ; n=8) subclinical mastitis groups. Each point and vertical bar represents the mean±SE of 7 and 8 animals.
IV

Abstracts Heifer Mastitis Conference
Prevalence and incidence of clinical and subclinical heifer mastitis

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Mastitis in heifers, primiparae, has always seemed to be less prevalent than in multiparous cows. Milk somatic cell count (SCC) is not appreciably affected by days in milk for heifers, differing with cell count changes in multiparae that increase with stage of lactation. Initially milk somatic cell counts at first sampling, within 30 days of parturition, are very similar for heifers and multiparae. Yet the milk somatic cell count is not appreciably affected by days in milk for heifers, which differs with cell count changes in multiparae which increases with increasing stage of lactation. Moreover, the lactation mean SCC is lowest for primiparae, and increases with each lactation. Milk SCC dynamics for heifers relative to multiparae suggest that mastitis is not a substantial problem. The symposium will reveal, heifer mastitis is significant, resulting in lost production, decreased profit, which necessitates intervention strategies designed to reduce the prevalence and incidence of the disease. The thrust of this discussion will be the prevalence and incidence of bovine heifer clinical and subclinical mastitis, at parturition and during first lactation.

New intramammary infections (IMI) in heifers will occur at least as early as breeding age. Fox et al. report that 34.6% of 1583 heifers (8-38 mo of age) had IMI. Of these infections, 78.3% were deemed to be caused by coagulase negative staphylococci (CNS). At parturition, 64% of heifers had IMI of which 60.5% were caused by CNS. Pankey and coworkers found 54.4% of heifers had IMI at parturition, 52.3% were a result of CNS IMI. In another study, 76.5% and 60.3% of IMI prepartum and postpartum in heifers were caused by CNS.

The prevalence of mammary quarters with CNS IMI is approximately twice the prevalence in multiparous cows. The incidence of CNS IMI decreases with increasing days in milk and parallels that observed in multiparous cows. Duration of IMI postpartum appears to be significant. Although CNS IMI prevalence decreased with advancing lactation, the duration of IMI over lactation was 67 days for primiparae, substantially higher than that reported where it was suggested that approximately 20% of IMI were eliminated during the first two weeks of lactation. Although the CNS are considered minor pathogens and are the predominant agents causing IMI in heifers pre- and postpartum, the major pathogens, perhaps most notably Staphylococcus aureus, can be a particular problem and constitute a significant fraction of all heifer IMI.

Heifers can show signs of clinical mastitis both pre-and postpartum. Whereas two thirds to three fourths of heifers have subclinical mastitis at parturition, the number of heifers with clinical mastitis peripartum is much lower. Kalmus et al. report 6.1% of heifers freshen with clinical mastitis and Pankey et al. reported an 8.1% prevalence at calving. Historically the focus on clinical heifer mastitis was studies on the disease complex termed “Summer Mastitis”. A review describes summer mastitis as an acute, suppurative infection affecting non-lactating animals, both dry cows and heifers. Arcanobacterium pyogenes and the anaerobe Peptococcus indolicae are frequently isolated. Additionally, Stuart-Schwan cocci, Streptococcus dysgalactiae and strict anaerobes: Fusobacterium necrophorum and Bacetetoboides melaninogenicus are also often associated with this complex. Summer mastitis appears to be most prevalent in northern European countries. However, even in these countries less than 25% of all clinical mastitis in heifers is a result of summer mastitis.
Heifer Mastitis Conference

The causative pathogens associated with the majority of clinical heifer mastitis are those that often are associated with clinical mastitis in multiparae. A report of 67.6% of clinical IMI for heifers at calving were environmental streptococci, 10.8% for CNS, and 2.7% coliforms. No heifer clinical mastitis infections by S. aureus were reported at parturition, in contrast to 15.4% of heifers with S. aureus clinical mastitis in the following lactation. Other first lactation clinical mastitis in this study was 38.5% environmental streptococci, 23.1% coliforms and 7.7% CNS. A study reported a much higher prevalence of clinical mastitis by S. aureus (44.2%) during the immediate prepartum or day of parturition, with Streptococcus dysgalactiae and CNS clinical IMI at 19.6% and 11.6%. Compared with these subclinical infections, similar percentages of clinical IMI by pathogen type during the first 14 days postpartum were found.

Most of the clinical mastitis in heifers occurs within the first 30 days postpartum, perhaps chronologically closer to parturition. Jonsson et al. reported that 76% of clinical heifer mastitis occurred during the first 7 days postpartum. Barnounin and Chassagne indicate that 67.7% of clinical heifer mastitis occurred during the first 30 days in milk. Another report indicates that the risk of clinical mastitis during the first lactation is lowest in primiparae and increases with increasing parity. Additionally they report that for heifers, the risk is highest within the first 15 days, higher than second lactation cows and decreases thereafter. This is similar other studies.

Although heifer mastitis seems to be less prevalent than mastitis in older cows during lactation, it is still a significant prepartum and postpartum disease. More primiparae than multiparae have subclinical mastitis, but that can be accounted for by the increased prevalence in CNS IMI in heifers. Clinical mastitis may actually be more prevalent in heifers than older cows, peaking within the first 30 days postpartum. During the remainder of lactation it is lower than that for older cows. A large majority of clinical mastitis in heifers is caused by major mastitis pathogens, in contrast to subclinical IMI.

References:
the somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80:3219-3216.


Prevalence and incidence of (sub)clinical mastitis in heifers in a random sample of dairy herds in the Netherlands.

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Introduction: Mastitis is of great importance for the future of the production life of a heifer. It causes, amongst others, a reduced production3 and an early culling of the infected animals2. Mastitis in heifers occurs less frequently compared to multiparous cows, although a higher incidence is observed in the first part of lactation1,2. Despite the knowledge about the effects of heifer mastitis, no recent estimates of occurrence of (sub)clinical mastitis are known in the Netherlands. Therefore, a survey was conducted to estimate the prevalence and incidence rate of (sub)clinical mastitis in heifers.

Materials and Methods: A survey on 396 dairy farms, randomly distributed in the Netherlands, was conducted from July 2004 until June 2005. Farms had to have > 50 cows and they had to participate in the regular test day recording, with test day intervals of 3 – 6 weeks. Composite somatic cell counts (CSCC) of all 16,572 heifers on these 396 herds were gathered from the regular test day recording to estimate prevalence and incidence of subclinical mastitis (SCM). SCM prevalence was calculated as the proportion of heifers with a CSCC > 200,000 cells/ml. SCM incidence was calculated as the number of new infections (an increase in CSCC > 200,000 cells/ml after being two consecutive test days ≤ 200,000 cells/ml) divided by the number of heifer days at risk. Clinical mastitis (CM) in heifers was additionally recorded by the farmer in a subset of 9,850 heifers on 205 farms. CM incidence rates were calculated as the number of quarter cases divided by the number of heifer days at risk. Negative binomial models were used to estimate the prevalence and incidences of (sub)clinical heifer mastitis.

Results: SCM prevalence in heifers (CSCC > 200,000 cells/ml), was on average 13.2% [12.6-13.9] from July 1st, 2004 until June 30th, 2005 and being the highest in August 2004 and the lowest in January 2005 (Figure 1). The percentage of heifers with 1 or more SCM infections was on average 27.2% per farm, resulting in an incidence rate of 0.806 cases per 365 heifer days at risk. CM was recorded in 8.1% of the heifers with an average of 0.191 cases per 365 heifer days at risk (Table 2). This was higher compared to the study of Barkema et al. (1998) in which CM incidence rate was 0.160 cases per 365 heifer days at risk, although this is probably not a significant difference.

Conclusion: This study gives insight in the prevalence and incidence of (sub)clinical mastitis in Dutch heifers and it shows that mastitis in heifers is an important issue in dairy practice in the Netherlands.

References:

Figure 1: Prevalence of subclinical mastitis in Dutch heifers based on composite somatic cell counts of 396 dairy herds from July 1st, 2004 until June 30th, 2005. Subclinical mastitis was defined when composite somatic cell counts were > 200,000 cells/ml.

Table 1: Number of herds and heifers in the study, the percentage of heifers with ≥1 infections and the incidence rate for both subclinical and clinical mastitis from July 1st, 2004 until June 30th, 2005. The 2.5- and 97.5-percentiles are between brackets.

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</tr>
<tr>
<td>Number of heifers in study</td>
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<tr>
<td>Number of infections</td>
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<tr>
<td>Percentage of heifers with ≥1 infections (%)</td>
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<tr>
<td>Incidence rate (/365 days at risk)</td>
<td>0.806 [0.765-8.849]</td>
<td>0.191 [0.171-0.212]</td>
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Incidence of mastitis and bacterial findings at acute clinical mastitis in Swedish primiparous cows – influence of breed and stage of lactation.

Karin Persson Waller1,2, Björn Bengtsson1, Ann Lindberg3, Ann Nyman2,3, Helle Unnerstad1

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2Swedish University of Agricultural Sciences, Uppsala, Sweden
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Introduction: Investigating trends in mastitis at the national level is useful to initiate correct control measures. In Sweden, approximately 86% of the dairy cows are enrolled in the Swedish official milk recording scheme (OMRS). Only veterinarians are allowed to start an antibiotic treatment and every treatment should be reported to the Swedish animal disease recording system (ADRS). These two systems are linked giving unique possibilities for studies of the dairy population. In Sweden, the two main dairy breeds are Swedish Red (SR) and Swedish Holstein (SH). SR has been reported to have better udder health than SH when observing the whole cow population. Knowledge of the panorama of udder pathogens causing acute clinical mastitis in dairy cows is also important for the control of mastitis. The aims were 1) to study the occurrence of mastitis, measured by the incidence of veterinary-treated clinical mastitis (VTCM) and the milk SCC at monthly recordings, in Swedish primiparous cows in relation to older cows and differences between breeds, 2) to investigate differences in the distribution of bacterial findings at acute clinical mastitis between primiparous and older cows, and 3) to study the occurrence of VTCM and bacterial findings in relation to stage of lactation using data from several field studies.

Materials and Methods: All cows (n=340,235 to 380,340/year) included in the OMRS were used when studying occurrence of mastitis in primiparous cows (n=132,157 to 139,428/year) in relation to older cows and differences between breeds. Data on VTCM (number of diagnosed cases per 100 cows) and geometric mean milk SCC was collected for years 2002-2006 (Swedish Dairy Association, Stockholm). Data on days in milk for VTCM was collected from two recent Swedish field studies1,2. Milk samples were collected from 829 cases of acute clinical mastitis in 2002-2003, and bacteriological analyses were performed using accredited methods. Differences in distribution of bacteria between time periods were tested using Chi-square analysis.

Results: When studying all cows enrolled in the OMRS, the incidence of VTCM (Figure 1A) and the SCC (Figure 1B) were lower in primiparous than in older cows. In the same material, primiparous SR cows had lower incidence of VTCM (Figure 2A) and lower SCC (Figure 2B) than primiparous SH cows. According to the field studies, approximately 50% of all VTCM in primiparous cows occurred just before calving or during the first month of lactation, and a majority of those were found during the first week after calving. In total, 1014 bacterial diagnoses were obtained from acute cases of clinical mastitis where parity information was available. Of those, 38% (n=385) originated in primiparous cows. The most common pathogen isolated in both primiparous and older cows was Staphylococcus aureus followed by Streptococcus dysgalactiae and Escherichia coli. In primiparous cows, a significantly higher proportion of cases occurred 0 to 30 d after calving than 31-120 d after calving. In contrast, E. coli was significantly less common in early lactation than in later stages.
Conclusions: Approximately 10% of Swedish primiparous cows are veterinary-treated for clinical mastitis each year, and the geometric mean SCC of primiparous cows is approximately 65,000/ml. Primiparous SR cows have better udder health than SH cows indicating a stronger immune defence in SR cows. In primiparous cows, most clinical cases of mastitis occurred during the first weeks after calving, and S. aureus was the most common udder pathogen during this period. The results indicate that some of those cows were infected before calving, but that a majority of the infections occurred 7-30 d after calving. Thus, better control measures are warranted both during late pregnancy and early lactation to reduce the risk of mastitis in primiparous cows.

References:
SESSION 2: ASSOCIATED PATHOGENS AND SOURCES OF HEIFER
MASTITIS

012 Types and sources of mastitis pathogens associated with heifer
mastitis

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Summary
Intramammary infections (IMI) in breeding age and pregnant heifers are much higher than
previously thought (1, 2). Many of these infections can persist for long periods of time, may
be associated with elevated somatic cell counts (SCC), and may impair mammary
development during gestation and affect milk production after calving. Some IMI in heifers
result in clinical mastitis during the prepartum period and during early lactation. Presence of
IMI before calving increased risk of infection during lactation, IMI at calving increased the
risk of clinical mastitis within the first week after calving, and mastitis prior to parturition
and mastitis within the first week after calving increased the risk of further cases of mastitis and
culling during the first 45 days of lactation. One common denominator of all studies
conducted on heifer mastitis throughout the world is the high prevalence of coagulase-
negative Staphylococcus species (CNS) IMI. While CNS are often grouped together,
considerable variation in the frequency of CNS isolation between herds has been reported and
it is possible that some CNS may be more problematic than others. Thus, CNS will likely cause
the majority of IMI in unbred and pregnant heifers and variation in the prevalence of CNS IMI in
heifers should be expected among herds. A variety of different mastitis pathogens have been
identified from heifers with clinical mastitis during the prepartum period and during early
lactation. The purpose of this overview paper is to discuss the types and sources of mastitis
pathogens associated with mastitis in heifers.

Discussion
Mastitis in heifers was first recognized over 60 yr ago (1). However, it was generally
believed that intramammary infections (IMI) in unbred and pregnant heifers occurred
infrequently. During the last two decades, several studies on the prevalence of mastitis in
heifers have been conducted and reported in the literature. All of these studies suggest that
IMI in heifers during the prepartum and peripartum periods occur quite frequently. Many of
these infections can persist for long periods of time, may be associated with elevated SCC,
may result in clinical mastitis, and may impair mammary development during gestation and
affect subsequent milk production after calving. Results of this research have also shown
marked herd variation in both the rate of IMI and types of pathogens causing IMI in heifers
(1, 2).

One common denominator of all studies conducted on heifer mastitis throughout the world is
the high prevalence of CNS IMI. The prevalence of CNS in mammary secretions of
primigravid heifers during the prepartum period has been reported to be as high as 50% of
mammary quarters (1, 2). However, the importance of CNS IMI has not been clearly
delineated. The literature often refers to CNS as minor mastitis pathogens, which suggests that
CNS are non-pathogenic or mildly pathogenic. The designation CNS is used to include all
staphylococci and micrococci isolated from milk samples that are not Staphylococcus aureus.
As a rule, CNS are coagulase-negative, however, there are exceptions. The commonly
isolated CNS are part of the normal skin flora and include Staph. simulans, Staph. hyicus,
and Staph. epidermidis. In contrast, novobiocin-resistant species (Staph. xylosus, Staph.
saprophyticus, Staph. sciuri, and Staph. cohnii) are found free-living in the environment. The
CNS appear to be opportunists and infect the teat canal and gland from skin sources. Infections by novobiocin-resistant species may originate from the environment. *Staphylococcus chromogenes* and *Staph. hyicus* appear to readily colonize the teat canal and may persist for longer periods of time than the other CNS. Many CNS infections are transient and cow to cow spread is thought to be a low risk for infection. *Staphylococcus chromogenes* was isolated most frequently in separate studies. However, isolation of other CNS varied considerably. Thus, while CNS are often grouped together, considerable variation in the frequency of CNS isolation between herds has been reported and it is possible that some CNS may be more problematic than others. Based on studies conducted thus far, CNS will likely cause the majority of IMI in unbred and pregnant heifers and variation in the prevalence of CNS IMI in heifers should be expected among herds.

The prevalence of mastitis pathogens other than CNS also varies considerably. In our studies at The University of Tennessee, 8 to 10% of heifer mammary glands were infected by environmental mastitis pathogens, primarily *Streptococcus* species, which was consistent with the pattern of IMI in lactating cows in these herds (1, 2). Conversely, other studies reported that *Staph. aureus* was the most prevalent major mastitis pathogen isolated from unbred and pregnant heifer mammary glands. Differences in the incidence of IMI and types of bacteria causing IMI in pregnant heifers is likely due to the prevalence of mastitis pathogens in the herds evaluated. Thus, a reasonable hypothesis is that heifers from herds with a high prevalence of contagious mastitis will likely be infected predominantly by contagious mastitis pathogens. Similarly, environmental mastitis pathogens will likely be the predominant major pathogens isolated from heifer mammary glands from herds with an environmental mastitis problem.

Some IMI in heifers result in clinical mastitis during the prepartum period and during early lactation (1-3). Presence of IMI before calving increased risk of infection during lactation, IMI at calving increased the risk of clinical mastitis within the first week after calving, and mastitis prior to parturition and mastitis within the first week after calving increased the risk of further cases of mastitis and culling during the first 45 days of lactation. A variety of different mastitis pathogens have been identified including *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staph. aureus*, CNS, *Escherichia coli*, *Acinomyces* (Arcanobacterium) pyogenes, *Stuart-Schwan coccus* and strictly anaerobic bacteria such as *Peptostreptococcus indolicus*, *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*. A large study from Norway (3) evaluated 1,361 cases of clinical mastitis in 1,040 heifers that occurred prepartum or within 14 days after calving. Mastitis pathogens isolated most frequently from mammary quarters with clinical mastitis were *Staph. aureus* (44.3%), *Strep. dysgalactiae* (18.2%), *Staph. aureus* together with *Strep. dysgalactiae* (1.2%), CNS (12.8%), *A. pyogenes* (3.5%), *A. pyogenes* together with *Strep. dysgalactiae* (0.5%) or *Staph. aureus* (0.4%), and *Escherichia coli* (6.4%). Of the CNS isolated, *Staph. simulans* (53.7%), *Staph. hyicus* (14.8%) and *Staph. chromogenes* (14.8%) were the most prevalent species.

References
Experimental model of bovine clinical mastitis caused by Staphylococcus chromogenes

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Introduction
A new experimental mastitis model was developed, in which a strain of coagulase-negative staphylococcus (CNS) S. chromogenes was used to induce udder infection. The aim of this study was to investigate the development of the disease and inflammatory reaction of the host to mastitis caused by a minor pathogen, CNS.

Materials and methods
Six clinically healthy Holstein-Friesian dairy cows in first lactation, with a low somatic cell count (SCC) in their milk, were used in the study. The experimental challenge was done in early lactation. One udder quarter of each cow was inoculated using a CNS strain (S. chromogenes) isolated from subclinical mastitis in Finland. The infection dose was 2x10^6 colony forming units (CFU) per quarter in 5 ml of saline. The infection dose was selected based on preliminary challenge studies with different doses of the bacterial strain. One cow was excluded from the study after challenge, due to mastitis in another quarter. The cows did not receive any medical treatment. Before the challenge and at regular intervals after it the cows were examined clinically using a scoring system. Clinical status consisted of general attitude, appetite, temperature, rumen function, udder palpation and milk appearance. Milk samples were taken for bacteriological culturing, SCC, N-Acetyl-D-Glucosaminidase (NAGase) activity, and acute phase protein determination, and blood samples for acute phase protein determination. Serum amyloid A (SAA) was determined from blood and milk using a commercial kit (Tridelta Development, Wicklow, Ireland). The detection limit of bacteriological culturing was 10 cfu/ml.

Results
All the cows became infected with S. chromogenes. Clinical signs were mild and none of the cows showed systemic signs like fever. Only mild local signs in the inoculated quarter such as slight swelling and a few clots in the milk could be seen, which disappeared within the experimental period of one week. Milk decrease of the challenged quarter was on average 16.3 % (range 7.4%-27.3%) from the milk yield before challenge. The infection was eliminated within two days in all cows, except one cow which developed chronic mastitis. Somatic cell count increased in the affected quarters, peaking at 30 h post challenge (PC) to the median value of 2.39 x 10^6 cells/ml. After that SCC decreased substantially and then started to fluctuate until the 7th day, when median SCC was under 150 000 cells/ml. The SAA concentration in serum increased, but the peak levels at 48 h PC varied largely between the cows (19-131 mg/l). Serum amyloid A concentration in the milk before challenge was under the detection limit 0.3 µg/ml, but started slightly to increase at 22 h after the challenge (median 1.6 mg/l). The maximum value of SAA in milk was 13.94 mg/ml at 52 hours PC. The amount of SAA in milk varied strongly between morning and evening milkings, and was higher in the mornings than in the evenings.

Conclusions
Bovine experimental CNS mastitis has not been described before. A large inoculum dose was necessary to induce mastitis. Clinical signs were mild and SCC decreased back to the previous level in seven days. Serum amyloid A in milk behaves differently in mild mastitis.
caused by *S. chromogenes* than previously described in severe mastitis caused by *E. coli*, where the time depending variation is not seen [1]. Results from this study confirmed the mild character of CNS mastitis.

**References**

**Figure 1:** Mean bacterial growth and SAA in milk. Serum amyloid A content in milk elevated slowly and varies regularly depending time of the day

**Figure 2:** Mean bacterial growth and somatic cell count. The mean SCC value was over $0.15 \times 10^6$ cells/ml at 8 hours PC.
014 Frequency of teat canal closure in prepartum heifers and its relation to udder health

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Introduction
One of the most important economic issues in dairy farms is to reduce mastitis. Especially mastitis in the first lactation reduces the life achievement of milk cows and leads to massive replacement costs in a herd. Younger investigations showed that an increase of the somatic cell count is directly related to milk loss of 0.13 kg per day of the lactation per natural logarithm step (De Vliegher et al., 2005). The premature loss of the keratin plug of the teat canal seems to be a major risk for heifer mastitis (Williamson, 2002). The aim of the study was to evaluate this risk factor in a limited animal population under field conditions and to describe the relationship between open teat canals, infected udder quarters and mastitis cases in the first lactation in Lower Saxony.

Materials and Methods
Within the scope of a longitudinal study, 84 milk cows from six high-yielding dairy herds (> 9,000 kg) were examined. The clinical investigation of each heifer started 10 weeks ante partum. Secretion samples for microbiological tests were taken from open udder quarters. Sampling was repeated until parturition at min. 4-week intervals. After calving, double samples of quarter foremilk were taken for cytobacteriological analysis in at least 10 week intervals up to the end of the first lactation, as well as when clinical mastitis cases occurred. Farms with heifer mastitis problems were selected for the study. A heifer mastitis problem was defined when 50% of the heifers had a cow-level somatic cell count > 100,000 cells / ml. Mean bulk milk somatic cell count amounted to 184,000 cells/ml at the beginning of the survey. Study animals had to be clinically healthy and pregnant on the initial phase of the study.

Results and Discussion
A massive increase of open teat canals was detected in the preparturition period. No open teat canals appeared up to the 80th day before parturition, but 60% of the quarters had already opened on the 60th ante partum. From this time the number of open quarter teat canals continuously increased until birth (Fig.1). In contrast to the findings of Williamson (2002), our data show that the opening of the teat canals often occurred much earlier. Almost 50% of secretion samples from quarters with open teat canals showed bacteriologically positive results.

A time-scheduled infection pattern was observed, since during the days 100 to 60, 60 to 30, and 30 to 0 before calving, skin inhabitants (CNS, coryneform bacteria), cow-related, and environment-related micro-organisms prevailed, resp. (Tab. 1). Infections with CNS and with S. aureus persisted beyond the parturition. Quarters with microbiologically-positive results displayed a three-times higher risk of new infection with another bacteria in the following lactation than quarters with closed teat canals or with bacteriologically-negative findings in the secretion before parturition (RR = 3.0 CI95 (1.4-6.5)). 86% of all mastitis cases during early lactation and 73% of all mastitis cases of the lactation related to quarters with a teat canal open 10 days before parturition. In the course of the first lactation, the portion of quarters in “normal secretion” diminished further.

While the relative portion of udder quarters infected with CNS decreased during lactation, the importance of Sc. uberis continuously increased.
Conclusions
The available data show that open teat canals can appear already a long time before parturition and coincide with microbiological infections that subsequently develop into clinical and subclinical mastitis cases of the first lactation.

References

Tab.1: Bacteriological positive findings in the temporal course (%)

<table>
<thead>
<tr>
<th>Exam. Date</th>
<th>CNS</th>
<th>corynef. Bact.</th>
<th>Sc. uberis</th>
<th>S. aureus</th>
<th>Colif.</th>
<th>Other</th>
<th>Inf. Quarters (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D100-60</td>
<td>83</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74</td>
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<tr>
<td>D60-30</td>
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<td>7</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td>121</td>
</tr>
<tr>
<td>D30-0</td>
<td>77</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>212</td>
</tr>
<tr>
<td>PP</td>
<td>74</td>
<td>0</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>PP+10</td>
<td>71</td>
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<td>12</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>PP+20</td>
<td>69</td>
<td>3</td>
<td>13</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>PP+30</td>
<td>61</td>
<td>5</td>
<td>15</td>
<td>13</td>
<td>3</td>
<td>3</td>
<td>59</td>
</tr>
</tbody>
</table>

Fig.1: Development of open teat canals and bacteriologically positive quarter secretions ante partum (n = 84 cows, 336 quarters)
SESSION 3: PATHOPHYSIOLOGY AND IMMUNITY RELATED TO HEIFER MASTITIS

015 Cumulative Physiological Events Modulate the Inflammatory Response of the Bovine Udder to Escherichia coli Infections Around Parturition

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A high proportion of intramammary coliform infections present at parturition develop disease characterized by severe inflammatory signs and sepsis during the first 60 to 70 d of the cow’s lactation.

In the lactating bovine mammary gland, the innate immune system plays a critical role in determining the outcome of these infections. During the last two decades, research has increased significantly on bovine mammary innate defense mechanisms in connection with the pathogenesis of coliform mastitis. Opportunistic infections with coliforms occur when the integrity of the host immune system is compromised by physical and physiological conditions that make the host more susceptible. The innate immune system of many periparturient cows seems to be immunocompromised. The cow’s defense system is unable to modulate the complex network of innate immune responses, leading to incomplete resolution of the pathogen and the inflammatory reaction. Periparturient immunosuppression is unlikely to be the result of a single physiological factor; more likely, several entities will be found to act in concert, with profound effects on the function of many organ systems of the periparturient dairy cow.

Neutrophils are key effector cells of the innate immune response to intramammary infection, and their function is influenced by many physiological events that occur during the transition period. During the last 30 yr, most efforts have been focused on neutrophil diapedesis, phagocytosis, and bacterial killing. How these functions modulate the clinical outcome of coliform mastitis, and how they can be influenced by hormones and metabolism has been the subject of intensive research worldwide and is the focus of this review. The afferent (sensing) arm of innate immunity, which enables host recognition of a diverse array of pathogens, is the subject of intense research interest and may contribute to the variable inflammatory response to intramammary infections during different stages of lactation.

Based on cumulative research identifying different factors that contribute to periparturient immunosuppression, the development of novel interventions that modulate the inflammatory response or enhance effector cell function or both may offer some promise for preventing or treating IMI in periparturient cows. Two main approaches can be used to modulate the innate
resistance of the udder. The first is to enhance the efficacy of the efferent arm of innate immunity, that is, modulation of the attraction of competent phagocytes. It is generally accepted that the ability of neutrophils to ingest and kill bacteria is pivotal for the control of IMI. The second is to enhance fast detection of pathogens in the teat and udder cistern (afferent arm of innate immunity). Recognition of microbial molecular patterns by Toll-like receptors and other signaling pathways contribute to fast recognition. These receptors enhance expression of cytokines, in turn amplifying host responses to the pathogen. Such therapies must be achieved without causing exuberant inflammation that ultimately injures the mammary gland and increases mortality of the host.

At the moment, many mastitis research groups are searching to enhance resistance of the udder by enabling the mammary epithelium to secrete immune or antibacterial self and nonself (foreign) proteins. For example, proof of concept has been obtained with transgenic mice and cows. Interest is focused on, for example, lysostaphin (a prokaryotic protein that has potent anti-staphylococcal activity), lysozyme, lactoferrin, and soluble CD14. Inserting the gene for CD14 into the mammary gland will provide the CD14 necessary for recruitment of neutrophils and elimination of invading coliform mastitis-causing pathogens. Transgenic application of immune or antibacterial proteins is expected to unveil candidate genes whose promoter elements will enable temporal expression patterns. These genes could eventually be expressed through administration as food additives (certain fatty acids, for example, are known to be very active at the level of gene expression). Theoretically, inflammation-inducible expression constructs are superior to constitutive expression because the antibacterial proteins are only provided when needed.

However, most of the above mentioned novel interventions modulating the inflammatory response are still at an experimental stage. Meanwhile, the easiest prophylaxis today to prevent periparturient toxic mastitis is to provide periparturient cows not only with optimal hygiene conditions, but also with appropriate diets during the transition period and as few additional stress events as possible around calving. The metabolic demands of increasing milk secretion (protein and energy) affect the ability of the periparturient cow to manage its metabolism, as well as its ability to recover from an immunocompromised condition. Many theories exist on how better to manage the metabolic changes in the transition cow. Central to all these theories is to maximize feed intake and minimize serum NEFA levels around calving to maximize the profitability of the transition cow.

Key Words: Escherichia coli • periparturient • mastitis • cow
**016 In vitro growth of Staphylococcus aureus in primiparous and multiparous blood and milk neutrophils during early lactation**

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**Introduction:** Bactericidal activity of neutrophils in dairy cows is recognized as one of the major defense mechanisms in protecting mammary gland from infections. These cells have enormous potentials to eventually kill engulfed bacteria1,2,3,4. Little study has been conducted about this topic in the bovine. In this experiment the effect of parity on the neutrophils bactericidal suppression that occurs during early lactation was studied. The bactericidal activity was studied both in blood and milk neutrophils. Since Staphylococcus aureus (S. aureus) is a common cause of mastitis in dairy cows, blood and milk neutrophils bactericidal activity against this bacterium was investigated, during maximal immune suppression, early lactation1,2,3.

**Materials and Methods:** Primiparous cows (first gestation, 2.4±0.5 yr, n=6) and multiparous cows (forth to fifth gestation, 5.8±0.7 yr, n=6) were used. After calving (until 35±6 days after parturition), 1.5 L mixed cisternal quarter milk samples and 40 mL blood samples were aseptically collected3. To compare blood and milk parameters between the primiparous and multiparous cows, the two groups were sampled, and neutrophils were isolated from blood and milk accordingly3, 4, 5. The killing of the S. aureus, Newbould 305 strain, was monitored by a bactericidal assay using sample cultivation, accordingly2. Briefly, 100 µl live bacteria (5.107 / ml) were added to 500 µl viable neutrophils (5.106 / ml) isolated from blood and milk, or to 500 µl of HBSS with no neutrophils; they then incubated for 1 hour, and finally serially diluted2. After dilution and plating out, they were mixed with a sterile plastic loop in duplicate onto Columbia sheep blood agar (Biokar Diagnostic, Beauvois, France). The plates were incubated overnight at 37°C and colony counts were performed2. Results from the bacteriological assay are expressed and compared as the percentage of killed (% killing) S. aureus2.

**Results:** According to this study, the percentage of killed S. aureus by blood neutrophils was significantly higher than that of milk neutrophils (Figure 1). Compared to multiparous group, the bactericidal activities of blood neutrophils against S. aureus were 42.3 ± 3.4 % in primiparous (P < 0·01) and 23.2 ± 1.7 % in multiparous. Milk neutrophils killed only 10.2 ± 1.3 % S. aureus in multiparous (P < 0·01), 20.7 ± 2 % in primiparous cows (Figure 1). Our results confirm an impairment of blood and milk-resident neutrophils bactericidal activity against S. aureus at the onset of lactation, which was less pronounced in heifers.

**Conclusion:** Based on our previous3,4,5 and current studies, the higher growth of S. aureus in neutrophils from multiparous lactating cows was mainly associated with a less efficient intracellular free radical production resulting from protein kinase C and NADPH-oxidase dysfunction as well as from partial impairment of myeloperoxidase activity. The pronounced reduction in neutrophils bactericidal activity in multiparous cows may be involved in the underlying mechanisms that make these animals more susceptible to periparturient infectious diseases, especially mastitis1. Further studies are in progress to explain the impaired milk neutrophils bactericidal activity around parturition.
Heifer Mastitis Conference

**References:**


**Figure 1:** Killing (%) of *Staphylococcus aureus* Newbould 305 after incubation with neutrophils isolated from blood (solid bars) and milk (open bars) of healthy primiparous and multiparous cows during early lactation. The comparison is between primiparous and multiparous cows. Values are means ± SEM of 6 cows.
Heifer and quarter level factors associated with periparturient blood and milk neutrophil viability and concentration

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Introduction: It is generally accepted that neutrophils play an important role in the first line immune defense of the mammary gland. Reduced neutrophil viability in blood and/or milk may partly explain the high prevalence of intramammary infections (IMI) in dairy heifers in early lactation. Knowledge about factors associated with blood and milk neutrophil viability, however, is scarce. The objective of this field study was therefore to identify factors both at the heifer and quarter level associated with periparturient blood and milk neutrophil concentration and viability with in a large field study.

Materials and Methods: A total of 83 dairy heifers from 19 Belgian dairy herds were included in the study. Approximately one week before the expected calving date, blood samples were taken to determine neutrophil viability and selenium concentration. At the same time, body condition and cleanliness of the heifer were scored visually based on published scoring systems, and the presence of udder edema, teat end lesions and teat skin lesions were recorded. Also, teat apices of each heifer were sampled to determine whether colonization with non-aureus staphylococci (NAS) was present. Body condition was scored again at 5 to 8 days in milk (DIM). Blood and quarter milk samples were collected at 1 to 4 DIM to determine the neutrophil viability. Quarter milk samples taken at 1 to 4 DIM were cultured according to NMC standards. Data on different heifer level variables (age at calving, season of calving, supplementation with minerals/vitamins, DIM, admittance to pasture, and contact with lactating cows before calving) were collected. The outcome variables “% viable”, “% apoptotic”, and “% necrotic” blood and milk neutrophils, and the concentration of milk neutrophils were determined by flow cytometry. Associations between the normalized outcome variables and the different independent variables at heifer and quarter level as fixed effects were studied using multilevel, multivariable regression models with “herd” and “heifer” as random effects (MLwiN 2.02). The proportion of variance in the outcome variables occurring at the different levels was calculated by fitting a two-level (blood neutrophils) and three-level null model (milk neutrophils), respectively.

Results: An overview of all univariable and multivariable associations between the different independent and outcome variables is presented in Tables 1 and 2. Also, 5.3% and 94.7% of the variation in % viable blood neutrophils occurred at the herd and heifer level, respectively, whereas 5.8%, 43.6%, and 50.6%, and 12.5%, 38.9%, and 48.6% of the variation in milk neutrophil concentration and % viable milk neutrophils, respectively, occurred at the herd, heifer and quarter level, respectively.

Conclusions: We conclude that (1) variation in neutrophil viability mainly resides at the heifer and quarter level, indicating that future research should focus on heifer and quarter level rather than on herd level, (2) several heifer variables such as supplementation of minerals/vitamins, calving season, and age at calving are associated with neutrophil viability around parturition, and (3) teat apex colonization with NAS may generate new hypotheses in protecting the mammary gland.
References:

Table 1: Univariable and multivariable associations between heifer level variables and blood neutrophil viability.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Blood neutrophil viability</th>
<th>%viable</th>
<th>%apoptosis</th>
<th>%necrosis</th>
<th>%viable</th>
<th>%apoptosis</th>
<th>%necrosis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before calving</td>
<td>at 1-4 days in milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>uni° mult° uni mult</td>
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<td>uni mult</td>
<td>uni mult</td>
<td>uni mult</td>
<td>uni mult</td>
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</tr>
<tr>
<td>Blood selenium concentration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Admittance to pasture (yes/no)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Season of calving (April to June versus other months)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Body condition score after calving (&lt;4.0 versus ≥4.0)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Supplementation with minerals/vitamins (yes/no)</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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<td>Cleanliness of heifer (dirty versus clean)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Age at calving</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Udder oedema present (yes/no)</td>
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<tr>
<td>Contact with lactating cows (yes/no)</td>
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<tr>
<td>Calving place (stable versus pasture)</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

° univariable model: +/- = positive/negative association (P<0.2); = non-significant; ... = not tested
° multivariable model: +/- = positive/negative association; +/- (P<0.05); + +/- - (P < 0.01); + + +/- - - (P < 0.001); = non-significant

Table 2: Univariable and multivariable associations between heifer and quarter level variables and neutrophil viability and concentration. Neutrophil viability and milk concentration.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Neutrophil viability</th>
<th>Milk neutrophil viability</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>%viable</td>
<td>%apoptosis</td>
</tr>
<tr>
<td></td>
<td>uni° mult° uni mult</td>
<td>uni mult</td>
</tr>
<tr>
<td>Heifer level variables</td>
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</tr>
<tr>
<td>% viable blood neutrophils before calving</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>% apoptotic blood neutrophils before calving</td>
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<td>...</td>
</tr>
<tr>
<td>% necrotic blood neutrophils before calving</td>
<td>×</td>
<td>...</td>
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<tr>
<td>% viable blood neutrophils after calving</td>
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<td>+</td>
</tr>
<tr>
<td>% apoptotic blood neutrophils after calving</td>
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<tr>
<td>% necrotic blood neutrophils after calving</td>
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</tr>
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<td>Selenium blood concentration</td>
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<td>Supplementation with minerals/vitamins (yes/no)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Admittance to pasture (yes/no)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Cleanliness of heifer (dirty versus clean)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Season of calving (April to June versus other months)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Excessive udder edema (yes/no)</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Days in milk (first day versus day 2, 3 and 4)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Quarter level variables</td>
<td>×</td>
<td>+</td>
</tr>
<tr>
<td>Infection with major pathogen present at 1 to 4 days in milk versus culture-negative and NAS-positive</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Teat apex colonisation NAS (yes/no)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Teat end lesions (yes/no)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Teat skin lesions (yes/no)</td>
<td>×</td>
<td>...</td>
</tr>
<tr>
<td>Quarter position (left front versus other quarters)</td>
<td>×</td>
<td>...</td>
</tr>
</tbody>
</table>

° univariable model: +/- = positive/negative association (P<0.02); = non-significant; ... = not tested
° multivariable model: +/- = positive/negative association; +/- (P<0.05); + +/- - (P < 0.01); + + +/- - - (P < 0.001); = non-significant
° Non-aureus staphylococci
SESSION 4: IMPACT OF HEIFER MASTITIS: ASSOCIATION WITH SCC, PRODUCTION, CULLING, AND FERTILITY

018 Impact of heifer mastitis: association with somatic cell count, production, culling and fertility

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Introduction: A high proportion of dairy heifers freshen with one or more infected mammary quarters. Whether the presence of those intramammary infections (IMI) during gestation and in early lactation has an effect on future performances and to what extent, remain critical questions. It is anticipated that the magnitude of the effect is related to the time of onset of the infection and to the causative pathogen, its virulence, and its capability of persisting when milk production has started. The present paper summarizes the current knowledge on these subjects and suggests future research efforts.

Literature overview: Probably the first study revealing the significance of "heifer mastitis" reported that mammary tissue of infected quarters from unbred heifers was less developed and had more leukocyte infiltration compared with uninfected quarters. Also, secretions from infected mammary glands had higher somatic cell counts (SCC) than secretions from uninfected glands. This corresponds well with the finding that IMI at parturition generally results in an increased SCC at that moment. The presence of an IMI in unbred and primigravid heifers was hypothesized to impair mammary growth and development, compromising future milk production. The initial ranking of heifers based on SCC classes in early lactation (<100,000, 100,000 to 400,000, and >400,000 cells/mL) was maintained throughout the first and subsequent lactations, indicating a long-term effect. This finding was studied in more detail recently and revealed that heifers with an elevated SCC in early lactation on average had elevated test-day SCC and had more cases of subclinical mastitis (defined as an SCC ≥200,000 cells/mL) throughout first lactation. The SCC in early lactation was measured between five and fourteen days in milk (DIM) and was used as a proxy of udder health early post partum. Also, the incidence of clinical mastitis increased with an increasing SCC in early lactation, and heifers with a higher mean SCC in the first lactation were at a higher risk of clinical mastitis in the second lactation. Heifers with a first test-day SCC <100,000 cells/mL produced approximately 400 and 750 kg more than heifers with a first test-day SCC between 100,000 – 400,000 and >400,000 cells/mL, respectively. More recent, the average daily milk yield loss was estimated to be 0.13 kg for each unit increase in the natural log-transformed early lactation SCC. The estimated loss was larger when not accounting for test-day SCC, indicating that both impaired glandular development during gestation and a reduced mammary function throughout lactation, played a role.

Clinical and subclinical mastitis early post partum in multiparous cows have negative effects on reproductive performances. However, in heifers the association between udder health at calving and fertility has never been studied in detail, but it is anticipated that the effects are comparable and should not be minimized.
The culling decision process is complex and very farmer-, herd- and time-specific. Still, nearly 11% of heifers that were treated for clinical mastitis before calving or within the first two weeks of lactation were culled within one month after treatment. The main culling reason of 96% of these heifers was mastitis. Although udder health problems were the culling reason for only 10% of the culled heifers in another study, it was shown that heifers with an elevated SCC early post partum were more at risk of being culled in their first lactation for whatever reason compared to heifers with a lower SCC. High-producing heifers, however, were protected from culling, whereas heifers with elevated test-day SCC later in lactation were more at risk.

An elevated SCC at 14 DIM had a larger effect on lactational SCC, milk yield and culling hazard than an equally high SCC at five days post calving. This finding indicates a pathogen-specific effect, related to transient (SCC in early lactation returning to normal within ten DIM, presumably caused by minor pathogens) versus persisting (SCC remaining high, presumably caused by a major pathogen) IMI. This is substantiated by the fact that IMI in heifers caused by minor pathogens in early lactation had no effect on SCC or on milk production during early to mid lactation. It also coincides with the belief that CNS-infections in general are cleared in early lactation because of their transient nature and that teat canal colonisations and subclinical IMI with CNS do not result in chronic infections and will not impair mammary function.

Conclusions: Udder health problems in dairy heifers in early lactation surely incorporate a threat to udder health and production in the first and subsequent lactation(s). A pathogen-specific effect seems to be present although quantification on a large number of animals and herds remains to be performed. Results of such studies will help to direct the decisions on blanket prepartum treatment of heifers. Whether mastitis in heifers, both clinical and subclinical, negatively influences reproductive capabilities still has to be studied. Moreover, whether time of onset of the infections during mammary development has an effect remains a question.

References:
**019 Influence of intramammary infections after calving on the risk of subclinical mastitis in the first 100 days of lactation**

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**Introduction.**  
The presence of intramammary infections (IMI) in heifer at calving has been reported by a number of studies. This result suggests to apply therapy before calving as a mean to reduce these IMI. The efficacy of these treatments showed to be generally high; however, their economic benefits are still under discussion. The aim of this study is to assess the prevalence of IMI in heifers after calving and if post-calving health status could be useful to predict the heifer udder health during the first 100 days of lactation.

**Materials and Methods.**  
Quarter milk samples (QMS) were collected in all calving heifers by aseptical procedure in the first 15 days after calving and then about every 3-4 weeks. Samples were cultured and colonies identified by proper methods according to National Mastitis Council (N.M.C., 1999). Somatic cells were counted on a Bentley Somacount 150 (Bentley USA).  
Quarter milk sample status was defined as follows: samples with SCC ≥ 200 000 cells/ml (either bacteriologically positive or negative) were defined as subclinical; bacteriologically negative samples with SCC <100 000 cells/ml were defined as negative, when the sample had SCC in the range 100-200.000 cells/ml (either bacteriologically positive or negative) was defined as diseased.  
To assess the pattern of SCC, data were analyzed using the MIXED procedure of SAS 9.1 including herd, cow and quarter health status as main factors, while the comparison of time from calving to subclinical mastitis outcome (days) associated to the quarter health status, was assessed by Kaplan-Meier survival analysis with LIFETEST procedure of SAS 9.1.

**Results.**  
Samples were collected in 25 herds following a herd health control program, which included the sampling of all animals in the first 2 weeks after calving, in addition to periodical samplings of all lactating animals in the healthy group. Overall 719 heifers were included in the study and 7868 QMS were collected. About 18% of the quarters were diseased or with subclinical IMI at calving and this value was maintained during the following period. More than 70% of the quarters were negative after the first sampling and until the end of the follow-up period (Table 1). CNS were the prevalent class of bacteria with an overall prevalence of 8.5% (47.7% of bacteriological positive results) (Table 2).  
To assess the relationship between quarter health status at first sampling and the outcome of subclinical mastitis during the first 100 days of lactation, quarters were classified in group H, if the first sampling after calving was negative, in group S when first sampling was defined as subclinical and in group D, if at first sampling it was diseased.  
The statistical analysis of SCC pattern during the follow-up period by health status of the quarter at first sampling showed that herd, time, quarter health status and the interaction between these last two factors were statistically significant. (P<0.001). Quarters in group S had mean SCC of 41,000 cells/ml, while group D had a mean of 12,000 cells/ml and H of 4,500 cells/ml. Statistical significant differences were always observed between group S and the other two groups when data were analysed by days in milk and health status, but not when group H and D were compared.  
The survival analysis showed that quarter health status at first sampling had a significant influence on hazard for subclinical mastitis (Figure 1). Indeed, time to subclinical mastitis was 50.5 days for group S, while it was respectively 88.3 and 95.7 for group D and H.
Conclusions.
The results of this field study confirmed that IMI can be observed in heifers at calving, with a prevalence below 20%, which is lower than reported in similar studies. At first sampling after calving, a similar proportion of heifers showed SCC counts >200,000 cells/ml independently of the presence of bacteria. These heifers showed significant higher SCC during the following trimester and nearly twice the rate of subclinical mastitis in the same period. These results support that blanket treatment of heifers prior to calving should be carefully evaluated based on a cost/benefit analysis, but they also suggest that sampling heifers after calving could be a useful procedure to identify animals at risk for subclinical mastitis.

Table 1: Distribution of bacteriological results (%) during the first 100 days of lactation

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Days in milk</th>
<th>CNS</th>
<th>Coliforms</th>
<th>Coryneb.</th>
<th>Env.Str.</th>
<th>Others</th>
<th>S.aureus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-14</td>
<td>8.4</td>
<td>0.6</td>
<td>0.1</td>
<td>3.0</td>
<td>5.8</td>
<td>0.3</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>8.3</td>
<td>0.7</td>
<td>0.6</td>
<td>3.2</td>
<td>3.6</td>
<td>0.7</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>31-50</td>
<td>7.8</td>
<td>0.4</td>
<td>1.1</td>
<td>4.5</td>
<td>3.5</td>
<td>1.3</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>51-75</td>
<td>8.6</td>
<td>0.2</td>
<td>0.6</td>
<td>2.6</td>
<td>1.4</td>
<td>1.1</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>76-100</td>
<td>10.7</td>
<td>0.8</td>
<td>0.7</td>
<td>4.8</td>
<td>2.5</td>
<td>1.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>8.5</td>
<td>0.6</td>
<td>0.4</td>
<td>3.3</td>
<td>4.3</td>
<td>0.7</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Table 2: Distribution of quarter health status (%) during the first 100 days of lactation

<table>
<thead>
<tr>
<th>Health status</th>
<th>Days in milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-14</td>
</tr>
<tr>
<td>Negative</td>
<td>65.9</td>
</tr>
<tr>
<td>Diseased</td>
<td>20.6</td>
</tr>
<tr>
<td>Subclinical</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Figure 1: Hazard function for subclinical mastitis outcome by quarter health status at first sampling (green line: group S; yellow line: group D; blu line: group H).
Prevalence and effects of mastitis pathogens isolated from dairy heifers in Argentina on milk production and lactation somatic cell count

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Introduction: Several studies have clearly shown that intramammary infections (IMI) in heifers occur frequently during the prepartum and peripartum periods (1, 4). However, limited information is available in Argentina (2, 3) and there is no data of mastitis pathogens effects on milk production and lactation somatic cell count. The purpose of this study was to determine the prevalence and effects of mastitis pathogens isolated from dairy heifers in Argentina on milk production and lactation somatic cell count (LSCC).

Materials and Methods: From January to December 2006 monthly individual somatic cell count (ISCC), milk production records and composite milk samples from 3,990 lactating heifers at 49 commercial dairy herds (ranged between 70 to 750 milking cows) of Argentina located in Province of Buenos Aires, Santa Fe, Cordoba and Entre Rios were collected. Isolates were identified according to the procedures of the National Mastitis Council. Differences between bacteriologically negative heifers and heifers with IMI on mean Kg 305-day lactation milk production and geometric mean LSCC were analyzed by Repeated Measures/General Linear Model.

Results: The results of bacteriological findings are summarized in Table 1. Table 2 shows the mastitis pathogens effects on milk production and lactation somatic cell count. The prevalence of IMI in composite milk samples from lactating heifers was 17.12%. One common denominator of all studies on heifer mastitis is the high prevalence of coagulase-negative staphylococci (CNS) IMI (2, 4). In the present study, CNS were the most commonly isolated bacterial species, followed by Staphylococcus aureus. The pathogens effects on milk production and LSCC showed significant differences for Staphylococcus aureus and Streptococcus spp., in comparison with bacteriologically negative heifers. Geometric mean LSCC for bacteriologically negative heifers, CNS, Staphylococcus aureus and Streptococcus spp. IMI was 54,000 cells/mL, 141,000 cells/mL, 303,000 cells/mL and 246,000 cells/mL, respectively. Mean Kg 305-day lactation was 150 kg, 425 kg and 300 kg lower for CNS, Staphylococcus aureus and Streptococcus spp. IMI, respectively in comparison with bacteriologically negative heifers. The total loss caused by Staphylococcus aureus and Streptococcus spp. IMI during lactation in comparison with bacteriologically negative heifers was 7.39 and 5.22%, respectively.

Conclusions: CNS were the most frequent isolates from composite milk samples of lactating heifers, followed by Staphylococcus aureus. The present study showed a significant influence of IMI by Staphylococcus aureus and Streptococcus spp., on milk production and LSCC. CNS had no statistically significant effects on milk production and LSCC, in comparison with bacteriologically negative heifers. The results indicate that milk loss in heifers depending on the pathogen responsible for the IMI. Staphylococcus aureus and Streptococcus spp. caused the greatest declines in milk yield and increases in LSCC. This study determined which specific mastitic pathogens are most detrimental for heifers milk production and LSCC.

References:


Table 1. Prevalence of intramammary infections (IMI) from 3,990 composite milk samples.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Composite n/(%)</th>
<th>Frequency n/(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>3,307 (82.88)</td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>141 (3.53)</td>
<td>141 (20.64)</td>
</tr>
<tr>
<td>CNS</td>
<td>348 (8.72)</td>
<td>348 (50.95)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>87 (2.19)</td>
<td>87 (12.74)</td>
</tr>
<tr>
<td>Other</td>
<td>107 (2.68)</td>
<td>107 (15.67)</td>
</tr>
</tbody>
</table>

Table 2. Mastitis Pathogens effects on milk production and lactation somatic cell count (LSCC)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Milk production*</th>
<th>LSCC (Log_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>5,750 ± 250</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>5,325 ± 200**</td>
<td>4.6 ± 0.2**</td>
</tr>
<tr>
<td>CNS</td>
<td>5,600 ± 150</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>5,450 ± 180**</td>
<td>4.3 ± 0.3**</td>
</tr>
</tbody>
</table>

*Mean Kg 305-day lactation
**P < 0.01
Heifer mastitis: it takes money

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Introduction: Mastitis is one of the most costly diseases in dairy industry (1). Mastitis problems don’t only occur in older lactating cows, heifer mastitis is also a well-known problem. Heifer mastitis potentially causes economic losses, caused by an elevated somatic cell count (SCC) at calving, and subclinical and/or clinical mastitis cases throughout lactation, which result in a decreased milk production, treatment costs, culling costs, and extra labour. Currently no data are available on the costs of heifer mastitis from scientific literature, besides some prepartum treatment effects (3). In this study we try to summarize the economics of heifer mastitis and calculate the costs associated with an elevated SCC at calving, and clinical and subclinical mastitis cases throughout lactation.

Materials and Methods: A tool, developed for farm specific calculations of mastitis in general (2), is used as base for the calculations for heifer mastitis. The calculation rules of this tool are converted into a stochastic model, in which the variation and uncertainty of heifer mastitis are taken into account. Input data like probabilities for infection (Pi), milk production losses (Pr), veterinary costs (Vet), cost of treatment and drugs (Tr), labour (Lab), losses associated with discarding milk (Dis) and culling (Cul) are based on literature search. The model consists of 2 parts. In the first part the dynamics of infection are simulated for elevated SCC at calving, subclinical, and clinical infections during lactation. In the second part the economic parameters are linked to the first part of the model and the total costs, costs per case, and costs per heifer present per farm are calculated. Figure 1 shows a graphical representation of the model.

Results: Using default values, the costs of heifers with an elevated SCC at calving are €17/heifer on average present on a farm (€0 - €185), consisting mostly of culling costs (€12) and production losses (€4). The cost of a clinical heifer mastitis case is on average €209, with production losses (€68), discarded milk (€17), and culling (€118) being the most important factors. This results in €14/heifer on average present on a farm (€0 - €180). The costs of subclinical mastitis are €25/heifer on average present on a farm (€0 - €250). These costs consist mostly of culling (€20), and production losses (€3). This results in total costs (elevated SCC at calving, clinical and subclinical infections) of €55/heifer on average present on a farm (€1 - €321).

Conclusion: Under default circumstances, the costs of heifer mastitis are €55/heifer present on an average farm with 15 heifers. The variation in these costs is very large (€1 - €321) and exists both within a farm and between farms. In the current approach long term effects are not taken into account, implying the total costs of heifer mastitis might be underestimated. Additionally, it’s important to know which part of these costs can be prevented. If we know the costs that can be prevented, this can be implemented in the advice and the farm management.
References:

Figure 1: Graphical representation of the model
Control of Heifer Mastitis I – Antimicrobial Treatment

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Replacement heifers are critical to dairy herd productivity because they represent the future milking and breeding stock of all dairy operations. The goal should be to provide an environment for heifers to develop full lactation potential at the desired age with minimal expense. Animal health and well-being play vital roles in achieving this potential, and one disease that can influence future productivity is heifer mastitis caused by Staphylococcus aureus and the coagulase-negative staphylococci (CNS).

Initial studies to determine the prevalence of mastitis in breeding age and pregnant dairy heifers demonstrated that intramammary infections (IMI) were present in 97% of heifers and 75% of quarters. The most common isolates were S. aureus, Staphylococcus hyicus, and Staphylococcus chromogenes; somatic cell counts (SCC) ranged from 12.4 to 17.3 x 10^6/ml. Approximately 29% of heifers and 15% of quarters exhibited clinical mastitis at breeding age as evidenced by clots, flakes, or blood in mammary secretions. Histologic examination of mammary parenchymal tissues from S. aureus-infected quarters demonstrated significant reductions in alveolar epithelial and luminal areas, and increases in connective tissue stroma and leukocytosis, illustrating limited mammary secretory development and marked inflammation of infected tissues.

A one-time infusion of nonlactating cow antibiotic preparations into infected quarters approximately 2 mo prepartum reduced incidence of IMI by 59% at calving compared with the pretreatment level; the cure rate for S. aureus IMI was >90%. Additionally, prophylactic treatment of uninfected quarters 2 mo prepartum reduced new Streptococcus spp. IMI by 93%. The mean SCC was 50% lower at calving for treated heifers, and milk yield over the first 2 mo of lactation was 10% greater than that of untreated controls. These early studies demonstrated that prevalence of IMI and SCC in dairy heifers were higher than previously realized, but that mastitis at calving was controlled by use of therapeutic products used for nonlactating cows during pregnancy.

In subsequent studies, dairy heifers were treated at 0 - 90 d, 91 - 180 d, or 181 - 270 d prepartum with 1 of 5 different antibiotic formulations to determine the best time and with which product heifers should be treated prior to calving. A total of 233 heifers was included in the study. At the initial sampling, 56.5% of quarters were infected with some type of organism, and 15.4% of quarters were infected with S. aureus. Nonlactating cow treatments compared were 1) cepahiprin, 2) penicillin-novobiocin, 3) penicillin-streptomycin, 4) an experimental product containing tilmicosin, and 5) a cephalonium product not available in the United States. In untreated controls, the percentage of quarters infected with S. aureus remained above 20% throughout most of the prepartum period, and decreased to 8.3% at calving. Among treated quarters, the percentage infected with S. aureus dropped below 5% after treatment and remained low through calving. Cure rates for the 5 antibiotic products indicated that all were effective (range: 70-100%) against S. aureus and all were significantly more efficacious than the spontaneous cure rate observed in untreated control quarters (range: 20-30%). No differences in efficacy were observed due to the different treatment times prepartum. However, fewer new S. aureus occurred after treatment in the group treated at 181 to 270 d prepartum, indicating that treatment in the third trimester of pregnancy will reduce the chances of new IMI occurring after treatment and persisting to
calving. Cure rates for the CNS and Streptococcus spp. were similar to those seen with S. aureus, and ranged from 80 to 100%.

More recently, prepartum lactating cow antibiotic therapy has been infused 1-2 wk prepartum to reduce the level of mastitis as well as SCC, and to increase milk production in first calf heifers. In a Tennessee study, 73% of Holstein heifers in a university research herd were found to be infected, and at ~2 wk prepartum, heifers were treated with a combination of penicillin/novobiocin, pirlimycin, or left as untreated controls. The majority of infections were caused by CNS (44%) and S. aureus (30%). After calving, quarter milk samples were taken to determine cure rates among treatments. Results showed that the spontaneous cure rate in controls was 26%; however, cure rate in quarters treated with penicillin/novobiocin was 76% and cure rate for those treated with pirlimycin was 59%. Frequency of isolation of mastitis-causing organisms in these heifers was followed over their first lactation. Results showed that in heifers treated prepartum, the percentage of quarters remaining infected was maintained below 20% for the duration of lactation, whereas in heifers left untreated, percentage of quarters infected was > 40% over the lactation.

Lactational performance and somatic cell scores (SCS) of antibiotic-treated and control heifers were also evaluated in this trial. For heifers receiving treatment (n=111), actual milk production (12,598 lb) was significantly greater than that in untreated controls (11,429; n=82). In addition, SCS was significantly lower in treated heifers compared with controls (2.04 vs. 2.63). Based on the increase in milk production in treated heifers, and based on the milk price at the time of the trial of $18.50/cwt, an average of $216.24 per heifer was realized in gross revenue. Considering the per heifer cost of teat hygiene ($0.10), antibiotics ($10.00), labor ($2.50), and residue testing ($3.00) for a total of $15.60, the adjusted increase in revenue per heifer treated was $200.64.

Results of these trials demonstrate that the treatment of heifers known to be at risk for developing IMI is advantageous because the cure rate is much higher than that obtained when treating infections during lactation. In addition, there is no milk loss, risk of antibiotic residue at calving is minimal, and future milk production is increased in heifers cured of IMI.
Heifer Mastitis Conference

023 Evaluation of the California Mastitis Test as a precalving treatment selection tool for Holstein heifers

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Corresponding author: jean-philippe.roy@umontreal.ca

Introduction: Mastitis in heifers has been well recognized for 15 years. During this period, many studies were conducted to evaluate the proportion of infected quarters and heifers, which reaching to 97% of heifers infected. Antibiotic treatments at many different intervals before precalving were also evaluated with very good results in order to decrease intramammary infection (IMI), including Staphylococcus aureus IMI, and sometimes increased milk production. However, the efficacy of those precalving treatments can be variable among herds. Also, there are some concerns expressed by veterinarians, producers and consumers about risks of antibiotic residue in milk and about rational antibiotic use. An approach targeting only infected quarters or infected precalving heifers would decrease the risk of antibiotic residue, theoretically decrease the risk of antibiotic resistance and decrease the treatment’s cost. An ideal diagnostic tool to select quarters or heifers for precalving treatment has to be quick, easy to do on-farm, reliable and cheap. Secretion appearance seems to be helpful to identify IMI but is not reliable enough.

The objective of this study was to evaluate California Mastitis Test (CMT) and a portable conductivity meter as diagnostic tools to assess precalving IMI in Holstein heifers and as a predictor of somatic cell count (SCC) and milk production at the first milk test day.

Materials and Methods: A total of 428 dairy Holstein heifers from 23 dairy herds in the St-Hyacinthe region were enrolled in the study. The participating herds were a convenience sample selected for willingness of the dairy producer to participate in the study, facilities to restrain heifers for sampling and geographical proximity of the farms to the researchers. Heifers were enrolled between 6 to 12 days before the expected calving date over a 12 month period from June 2002 to June 2003. Heifers could not have received an antibiotic during the previous three months and could not have been treated for a previous episode of mastitis. CMT was done on the farm before taking milk samples and results were noted as negative (N), trace (T), 1, 2 or 3. Then, duplicate milk samples were taken using aseptic technique from each quarter of all heifers. Two samples per quarter were taken when the quantity of secretion was sufficient. Milk samples were kept cool for transport and frozen within 12 h for later bacteriological analysis. The first milk sample was sent to the clinical bacteriology laboratory at the Faculté de Médecine Vétérinaire de l'Université de Montréal where the bacteriological analysis were performed according to NMC guidelines. Milk electrical conductivity was evaluated on the second of the duplicate samples using EC TesTr (Oakton instruments, 0 to 19.99 mS). This study was part of another study to evaluate the effect of precalving antibiotic treatment of Pirlimycin on Holstein heifers. In the initial study, 219 heifers were treated and 209 heifers were untreated controls. The precalving data from all heifers were used but only data coming from the 209 control heifers were used to analyse the effect on SCC and milk production at the first milk test day. SAS software v. 8.02 (Cary, N. C.) was used for all statistical analyses. For statistical analysis, CMT results were combine into 2 categories. CMT was considered negative if the initial result was N, T or 1 and was considered positive otherwise. Two cut-off points were evaluated for milk electrical conductivity (> 5 and > 6.5 mS). A quarter was considered...
negative above those cut-off points. Variables were considered statistically significant at $P \leq 0.05$.

**Results:** A total of 33% of quarters and 69% of heifers had precalving IMI. The most frequent isolates were coagulase-negative staphylococci (CNS) (59% of heifers), followed by *S. aureus* (10.3%). Gram-negative bacteria and yeast were encountered less frequently (3.5%). Overall, there was a poor association between the CMT results with the precalving IMI status of individual quarter (Kappa = 0.31). Many heifers (30.4%) had 3 or 4 quarters with a positive CMT. This is not a surprising finding because it is reported that average precalving SCC can vary from 3.5 x 10$^6$ c/mL to 6 x 10$^6$ c/mL on healthy quarters$^{1,8}$. On the other hand, 104 heifers (24%) had a negative CMT results for all 4 quarters. For those CMT negative heifers, CMT was a good predictor of no IMI with major pathogens (*S. aureus, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus spp, Arcanobacterium pyogenes, Califorms, Klebsiella spp and yeast*). From those 104 heifers, the CMT was able to correctly identify 97.6% of the heifers not infected with a major pathogen.

The mean precalving milk electrical conductivity was 6.5 mS (range 2.2 - 15.3) with a standard deviation of 1.9 mS. The milk conductivity had a very poor correlation with precalving IMI overall. The Kappa value was 0.09 and 0.05 when 6.5 and 5 mS were used as cut-off point respectively. Using a cut-off point of 5 mS, 178 heifers had negative milk conductivity for all 4 quarters. For those milk conductivity negative heifers, milk conductivity was a good predictor of no IMI with major pathogens. From those 178 heifers, the milk conductivity was able to correctly identify 96.5% of the heifers not infected with a major pathogen. With the cut-off level of 6.5 mS, 95.6% of the 244 negative heifers were correctly identified.

Precalving CMT and milk conductivity were poorly correlated with the SCC or the milk production at the first milk test post-calving.

**Conclusions:** The CMT and milk electrical conductivity are not good predictors of IMI for Holstein heifers in the last 2 weeks precalving. However, CMT and milk conductivity could be useful precalving to identify heifers not infected with a major pathogen in all 4 quarters. This represents 24% and 57% of the heifers when the CMT or the milk conductivity are used respectively. CMT or milk conductivity could be used as a rapid on farm tool in herd where precalving antibiotics treatment are given to all heifers to aim for a more targeted and possibly more economical approach.

**References:**

Periparturient use of micronised Procaine Penicillin to reduce the risk of mastitis in heifers

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Introduction: In New Zealand, periparturient heifer mastitis is recognised as both a significant welfare issue and of significant financial impact on commercial dairy farms. Mean reported incidence rates within the first week of calving for multiple herds within New Zealand vary, with reports highlighting rates of between 13%6 and 35%3. Within herds, clinical incidence rates may reach 61% in some herds9. Heifers mostly calve outside in spring on pasture, with large numbers of heifers calving over a short space of time. The predominant bacterial isolate is Streptococcus Uberis3. The effects of treating primaparous heifers within 18 hours of calving with a single dose of 15million iu of micronised Procaine Penicillin (Masticillin®) on subsequent mastitis incidence were investigated.

Materials and Methods: All heifers (n = 611) calving on 3 commercial dairy farms were examined at the first milking after calving and included for allocation to one of three groups. Those with any evidence of clinical mastitis at the time of initial examination (n = 3) were allocated to a direct treatment group and removed from the study. The remainder were randomly allocated to one of either control (n = 390) or treatment (n = 218) groups. Heifers allocated to the control group received no further intervention. Heifers allocated to the treatment group received one treatment of a single dose of 15million iu of micronised Procaine Penicillin (30mls Masticillin®) by intramuscular injections (2 separate injection sites). Primary outcomes of interest were: mastitis incidence in the first 7 days after calving (Mast7); mastitis incidence from 8-100 days after calving (Mast100); overall mastitis incidence days 1-100 (MastGen); and days to first case of mastitis. Secondary outcomes of interest were related to milk quality and quantity (MS production and SCC) and longevity and survivability.

Results: Fourteen heifers who calved before the beginning of the trial were removed from the analysis, leaving 376 in the control group and 218 in the treatment group. Heifers calved from 13/7/06 to 19/10/06, with a median calving date of 12/8/06. The overall incidence of mastitis in the first 7 days after calving (Mast7) was 7.2% (4.00%, 7.20%, 9.90% over the 3 farms). The Mast7 incidence in the treatment group was 4.1%; and in the control group was 9.00%. After adjusting for farm, treatment had a significant effect on the incidence of mastitis within 7 days of calving (p = 0.044); and reduced the odds of a heifer experiencing mastitis within 7 days of calving by over half (Mantel-Haenszel adjusted OR 0.456).

The overall incidence of mastitis between days 8-100 was 4.4% (Farm variation 0.7%, 6.40%, 4.40%). After adjusting for Farm, treatment had no effect on mastitis incidence between 8-100 days. The overall incidence of mastitis from calving to 100 days was 12.0% (Farm variation 4.7%, 14.4%, 14.4%). Treatment had a significant effect on the incidence of mastitis from calving until day 100 after adjusting for Farm (p = 0.027); and reduced the odds of mastitis by almost half (MH adjusted odds ratio 0.518). Days to first case of mastitis was analysed using both Kaplan Meier univariate screening followed by Cox proportional hazards analysis. Both Farm and treatment were significant using a Log Rank test and were both included in the final model. The final model showed treatment had a significant effect on the median time to mastitis (p = 0.019; β = 1.961; LCI 1.117, UCI 3.445).

Conclusions: It has been suggested that in many heifers who develop mastitis within 7 days of calving, infection may have originated as long as 9 months prior to calving5. Consequently, treatment before calving or immediately after calving may be expected to
remove any subclinical infection before it becomes clinical. Other authors have reported studies using intramammary products prior to calving\textsuperscript{2,4} but these have significant risks associated with their use in terms of introduction of infection and sphincter damage. Parenteral treatment has the benefit of being significantly less invasive and also allows all four quarters to be treated with one treatment. Previous work using Penicillin based products has shown a similar benefit of parenteral treatment prior to calving, but this has the negative effect of producing an antibiotic residue risk in the subsequent calf\textsuperscript{1,6}.

Effective treatment immediately after calving has the benefit of having no residue risk to an unborn calf, as well as fitting in with normal heifer management by not requiring an extra handling and yarding event, itself a risk in the development of mastitis. Treatment of a single dose of 15 million iu of micronised Procaine Penicillin (Masticillin\textsuperscript{®}) at the first milking after calving significantly reduced the odds of developing mastitis in the first week after calving to under half that of untreated controls. Treatment reduced the overall odds of developing infection until 100 days after calving, but this effect was largely driven by the significant reduction in mastitis incidence in the first 7 days. The median days to first mastitis was increased significantly ($p = 0.019; \beta = 1.961$)

References:

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2. McDougall S, Parker KI, Compton C, Heuer C. 2005 Reducing subclinical and clinical mastitis in dairy heifers by precalving infusion of a teat sealant and/or parenteral antibiotic therapy. Proc. 4\textsuperscript{th} IDF Conf 269-273.
Effect of periparturient treatment with penethamate hydriodide on the udder health of heifers in S. aureus problem herds

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Introduction: Intramammary infections in primigravid heifers is a relevant problem. Beside coagulase-negative staphylococci, one of the major pathogens, representing up to 37 % if IMI in heifers, is Staphylococcus aureus1. In a herd with a high prevalence of S. aureus infections, a mastitis control program should be implemented to deal with this contagious pathogen, including appropriate milking hygiene. Mastitis control programs do not usually involve heifers, even though heifers are representing the herd replacements, and consequently, the future of the milking herd. In the present field study we've evaluated the efficacy of parenteral treatment of heifers with penethamate hydriodide in S. aureus problem herds around parturition.

Materials and Methods: Seven Austrian dairy herds were selected based on following criteria: 1) At least 20% of cows per herd infected with S. aureus 6 months before the trial, 2) free stall housing, 3) minimum herd size of 30 cows, 4) Holstein Friesian and Simmental breeds, 5) annual milk yield between 6000 and 10000 kg, 6) practiced culling of chronically infected animals within six months of diagnosis and 7) introduction of a standardized milking management. The first 60 heifers that calved were randomly assigned to two groups: in the treatment group heifers received an intramuscular administration of 10 million I.U. penethamate hydriodide (Mamyzin®, Boehringer Ingelheim, Ingelheim, Germany) at the day of calving and 24 hours later 5 million I.U. Heifers in the control group were left untreated. The udder health was controlled by the California Mastitis Test and bacteriological examination of quarter milk samples 7, 14, 21, 35 and 49 days pp. All milk samples (0.01 ml) were inoculated onto Columbia blood agar and incubated aerobically at 37 °C for 24-48 h. A mammary quarter was considered infected if the same pathogen was isolated in two consecutive samples, two out of three samples, or a pathogen came from one milk sample with the cell count elevated in comparison to the other quarter cell counts. Individual milk yield data as well as SCC were recorded from each heifer the first 200 days of lactation in 5 weeks interval. Statistical analysis is based on Fisher’s exact test for frequency tables. For the comparison of the two treatment groups the non-parametric Wilcoxon test was used. Test of significant differences in changes to baseline was calculated using the signed rank test of Wilcoxon. Probabilities less than 0.05 were considered as significant. The analysis of milk production was made by modelling the milk yield in consideration of treatment group, breed, herd, and time. The per-heifer net revenue change from treatment was calculated by the following formula: \[ NR = p(Q^T - Q^U) - C^T - C^D \] where NR is the net revenue change from treatment, p is the price of milk, \( Q^T \) – \( Q^U \) is the difference in milk production for treated and untreated groups, \( C^T \) is the cost of treatment and \( C^D \) is the cost of discarded milk of day 6 pp.

Results: In total, 11 heifers showed intramammary infections during the first 49 days pp. The incidence of IMI was higher in the control group (25 quarters) than in the treatment group (9 quarters), however no significant difference could be observed. IMI in the control group occurred earlier in lactation with more severe and more persistent cases. In the antibiotic-treated group, no IMI were detected in the first week pp. and no mastitis case required treatment during the study period of 7 weeks pp. Subclinical S. aureus infections were...
observed 35–49 days pp. In the control group, infections were present in four heifers in the first week. Two heifers were affected with severe *S. aureus* infection and required immediate treatment. Both cases recurred as subclinical mastitis at subsequent samplings. Two moderate mastitis cases caused by streptococci had to be treated during the study period. One severe *E. coli* mastitis case occurred in the second week of lactation and had to be treated. No significant differences in SCC were detected between the two groups. Within each group, no significant changes from baseline were observed. Milk yield of antibiotic treated heifers was significantly higher within the first fifteen weeks compared to the control group (table 1). Therefore the net revenue yielded EURO 56.11 per heifer.

**Conclusion:** According to recent studies\(^1,2\) antibiotic therapy of heifers around parturition reduced new intramammary infections by killing invading bacteria during the peripartal period. In herds with high prevalence of *S. aureus* mastitis introducing periparturient treatment of heifers with penethamate hydriodide protects these animals, which represent the future of the milking herd, from intramammary infections and leads to higher milk production.

**References:**

**Table 1:** Median milk yield data (kg/day) of treatment and control group during 30 weeks of lactation.

<table>
<thead>
<tr>
<th>Weeks of lactation</th>
<th>group</th>
<th>1-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>26-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td>30.00*</td>
<td>30.00*</td>
<td>30.00*</td>
<td>26.50</td>
<td>25.40</td>
<td>24.40</td>
</tr>
<tr>
<td>Control</td>
<td>24.90</td>
<td>26.00</td>
<td>24.80</td>
<td>25.20</td>
<td>22.40</td>
<td>23.20</td>
<td></td>
</tr>
</tbody>
</table>

\(**P<0.05\)
Factors associated with the risk of antibiotic residues and intramammary pathogen presence in milk from heifers administered prepartum intramammary antibiotic therapy.

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Introduction: Intramammary (IMM) mastitis pathogens originating during the prepartum period in heifers can persist into lactation2. A reduction in the prevalence of mastitis pathogens in heifers with prepartum IMM antibiotic therapy has been reported; however, this therapy may result in residues in milk and compromise human food safety. Oliver et al.3 determined, in a small study, that antibiotic residues detected in milk at 3 days postpartum were reduced when heifers were treated at approximately 14 days (d) prepartum compared to 7 d before parturition, suggesting that a longer interval between treatment and parturition would reduce the risk for antibiotic residues occurring in milk postpartum. The association between the efficacy of the prepartum antibiotic treatment and the risk for antibiotic residues has not been previously established and has been evaluated in this study.

Materials and Methods: This was part of a study to determine the effect of prepartum IMM therapy on milk production, reproductive performance and cure rate of existing IMM infections1. Briefly, heifers from seven dairy herds in seven states (US) and two herds in one province (Canada) were assigned alternately by identification to one of two treatments at 14 d (range= 10 to 21 d) prior to anticipated calving date. At enrollment, treated heifers received commercial intramammary therapy of 200 mg of cephapirin sodium in each mammary quarter, controls were not treated. Milk was collected aseptically from all quarters immediately prior to IMM antibiotic infusion and at week (wk) 1, 2 and 3 postpartum and analyzed for mastitis pathogens. Interval (d) between prepartum antibiotic treatment and parturition and monthly milk production up to 4 months postpartum were recorded for all heifers. In a sub-set of herds, milk collected at the third, sixth and tenth milking postpartum from 136 treated heifers in five herds was analyzed for β-Lactam residues using a microbial inhibition antibiotic residue screening test. Antibiotic residues were confirmed with β-Lactamase treatment and re-tested for residues4.

Results: Antibiotic residues were detected in milk from 36 (26.5%) treated heifers at the third milking postpartum and 12 and 5 cows at the sixth and tenth milkings, respectively. Using logistic regression, greater intervals and milking numbers postpartum were associated with a decrease in risk for antibiotic residues4. Lower milk yields were associated with an increased risk for antibiotic residues in milk from the third milking postpartum (P < 0.001). Coagulase-negative Staphylococci (CNS) and Staphylococcus aureus were the most prevalent IMM pathogens and the presence of an antibiotic residue at the third milk postpartum (P = 0.04) and a shorter interval (P = 0.009) were associated with a decreased risk for isolating these pathogens in milk from parturition through 3 wk postpartum (Table 1).

Conclusion: Prepartum IMM antibiotic therapy administered at 18 d or less before parturition decreased the risk for isolating IMM CNS and Staph. aureus postpartum in heifers; however, this time interval was also associated with a small but significant increase in antibiotic residues in the third milking postpartum that diminished with time postpartum. These
results show that efficacy of prepartum IMM antibiotic therapy is confounded by a small increase in risk for antibiotic residues in milk postpartum and emphasize the importance of testing milk for antibiotic residues when antibiotics are administered prior to parturition.

References:

Table 1: Frequency and percentage of quarters isolated with no pathogens (0), or a total of 1, 2 and 3 pathogens in milk at 1, 2, and 3 wk postpartum from heifers categorized by the presence of antibiotic residues in milk at the third milking postpartum (antibiotic negative or positive) following prepartum antibiotic therapy and mean, SD, and range in interval in days (d) between prepartum antibiotic therapy and parturition.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of eligible quarters</th>
<th>Antibiotic negative</th>
<th></th>
<th></th>
<th>Antibiotic positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pathogen frequency</td>
<td></td>
<td>2 and</td>
<td>Pathogen frequency</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CNS</td>
<td></td>
<td>259</td>
<td>46</td>
<td>22</td>
<td>130</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td></td>
<td>(79.2%)</td>
<td>(14.1%)</td>
<td>(6.73%)</td>
<td>(90.3%)</td>
</tr>
<tr>
<td>Mean interval</td>
<td></td>
<td>303</td>
<td>10</td>
<td>14</td>
<td>137</td>
</tr>
<tr>
<td>d and (SD)</td>
<td></td>
<td>(92.7%)</td>
<td>(3.06%)</td>
<td>(4.28%)</td>
<td>(95.1%)</td>
</tr>
<tr>
<td>Interval range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>15.1 (3.81)</td>
<td></td>
<td></td>
<td>9.6 (3.88)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>7 – 25</td>
<td></td>
<td></td>
<td>1 – 18</td>
</tr>
</tbody>
</table>
SESSION 6: CONTROL OF (HEIFER) MASTITIS II - GENETICS

027 Genetic factors affecting susceptibility to udder pathogens

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Introduction
Using breeding as a measure to combat mastitis in dairy cattle has been the subject of considerable research effort over the past 20 years or so. Genetic studies have hypothesized two different modes of action for the genes underlying traits associated with mastitis resistance: the polygenic and major gene approaches.

The polygenic hypothesis
The polygenic hypothesis states that resistance is polygenic, i.e. due to the effects of a large number of genes. Under this hypothesis, heritability estimates have been computed for somatic cell count ($h^2 = 10\%$) and clinical mastitis ($h^2 = 5\%$), for heifers and multiparous cows. Currently, genetic evaluations for SCC in dairy cattle have become available in most countries because SCC is routinely recorded in most dairy cattle recording systems, contrarily to cases of mastitis. But there is still debate about decreased milk SCC as a measurement of resistance to infection and in the methods used to estimate SCC genetic parameters. Indeed, SCC are collected on a monthly basis and the infection status of the gland is usually unknown which may lead to biased estimates of genotypic values. Lowering SCC may also be inappropriate, since in some studies, milk SCC prior to experimental challenge with S. aureus was higher in cows that resisted infection than in cows that eventually became infected, with the reverse after challenge (Piccinini et al., 1999; Schukken et al., 1999). To address such issues, finite mixture and hidden Markov models were developed to provide tools to identify cows resistant to infection from resilient cows, i.e., cows able to oppose mastitis development (Detilleux, 2000, 2007). This is important as the long-term effects of selection on the dairy cattle for resistance or resilience may be different (Detilleux, 2005).

The h² estimates (0.26 to 0.36) for daily measures of electrical conductivity (automatic milking systems) from Holstein heifers suggest this indicator is a potential trait for breeding programs (Norberg et al., 2004).

Immunological assays have also been suggested as indicator of resistance. Reported h² may be high (ex. cells count, immunoglobulin, acute phase proteins, ..) but they vary with the type of assays and the time at which the assay is measured. Also, the assays cannot currently be used on the millions of cows registered in national breeding programs. To solve this issue, some authors have proposed to restrict the sampling on IA bulls (Kelm et al., 1997; Fitzpatrick et al., 1999) or to a few assays identified after evaluating their importance in the development of the response to udder pathogens (Detilleux, 2004; Detilleux et al., 2006).

The oligenic hypothesis
Under this hypothesis, it is stated that a few genes with major effects will confer resistance. For mastitis, quantitative trait loci (QTL) have been found on different cattle chromosomes but the greatest number are on chromosome 23, where the genes responsible for the major histocompatibility complex are located.

The most extensively studied MHC genes having significant associations with different indicators of mastitis are the MHC class II DRB3 alleles, some of which will confer increased or reduced resistance to mastitis. Recently, Rupp et al. (2007) reported associations between BoLA DRB3.2 alleles and immune responses tended to be in the opposite sign for traits
measuring antibody-mediated and cell-mediated immune responses, in support of the hypothesis that both traits are genetically independent and represent opposing type 1 and type 2 immune responses.

The advent of the ‘omics’ technologies will make possible the discovery of several of the key defense mechanisms that govern the resistance to mastitis at the molecular and genetic levels. Already, new candidate genes have been discovered such as the bovine forebrain embryonic zinc finger-like gene while others have been confirmed such as genes coding for IL-8 receptor, SAA3, toll-like receptors, CD14 and TNF-kB (Sharma et al., 2006; Shwerin et al., 2003; Sugimoto et al., 2006; Youngerman et al., 2004; Zheng et al., 2006). The immunological role of the mammary epithelium in the early stages of the interaction between pathogens and the host has also been stressed (Pareek et al., 2005). Because of the complexity, diversity and quality of data generated by these modern technologies, we have to bring together expertise from statistics, mathematics, bioinformatics, and computer science to handle such task (Seifert et al., 2007)

Conclusions
Evidence from immunology and genetics demonstrate selective breeding of genetically-based resistance is likely to modify the relationships between cows and pathogens, as reviewed above. The challenge is to integrate effectively the information from both fields into existing breeding programs. Further studies are necessary when one realizes current intensive selection for increased milk production may lead to selection of cows less resistant to infection due to undesirable genetic correlation between milk yield and mastitis frequency.

References


Genetic parameters for clinical mastitis in primi- versus multiparous cows

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Introduction: Clinical mastitis (CM) is one of the major diseases in dairy cattle. Mastitis control programs are often designed to provide guidelines to reduce CM on farms. Genetic selection is another strategy to combat CM. Although genetic selection is a slow process, it results in a permanent change in the genetic composition of the dairy herd. The (first) breeding value for udder health of a bull is based on the performance of his daughters in their first lactation. However, CM is not a problem in first lactation only. Actually, both frequency of CM, and level of somatic cell count (SCC) increase with increasing parity. Therefore, the aim of this study was to estimate genetic parameters for CM and lactation-average SCC for the first 3 lactations of Dutch Holstein cows.

Materials and Methods: Records on CM treatments were available from Management Information Systems on Dutch dairy farms. Data recording was done on a voluntary basis and was performed by the farmers themselves. Data on CM were recorded from June 1998 until May 2006, and all herds participated in the national milk recording system. The data was edited with criteria on age at calving, parity, lactation length, Holstein-Friesian% and availability of pedigree information. After editing the data, the final dataset consisted of 35,379 lactations from 21,064 cows on 250 farms. A pedigree file was constructed based on sires and maternal grandsires (MGS) of the cows in the final dataset, and contained 3,855 bulls with their parents.

Clinical mastitis was defined on a lactation basis as an all-or-none trait; either a cow had CM in a certain lactation (1) or not (0). Through this, only first cases of CM per lactation were taken into account. The cell counts were transformed to somatic cell score (SCS=1000+100*(\(2\log(SCC/1000)\))), and was averaged per lactation over test-day records between 5 to 335 days in milk (DIM). Also, SCS in the first half of the lactation (5-150) was compared with SCS in the second half (151-335).

ASREML was used to estimate variance components for CM and SCS using a linear sire/MGS-model. Univariate analyses were carried out for CM and SCS, with the following model: 

\[ Y = \mu + \text{fixed effects} + S_{\text{sire}} + \frac{1}{2} S_{\text{mgs}} + e \]

The random sire effect was identified by sire and MGS. The sire effects were linked using the relationship matrix, and were assumed to be normally distributed (\(\sigma^2_s\)). Fixed effects included were parity (3 classes), age at calving, an interaction between herd and year of calving (with 948 classes), and month of calving (12 classes).

Bivariate analyses were carried out to estimate genetic correlations between CM and SCS. The applied model for the bivariate analyses was the same as the one applied for the univariate analyses.

Results: The mean proportion of cows that had CM at least once during lactation was 15.8%, and was lowest for heifers (13.4%) and highest for 3rd parity cows (19.6%). Of all 2nd parity cows 16.1% got CM at least once during lactation. The proportion of heifers with CM increased rapidly up to 50 DIM (Figure 1). Actually, half of all first cases of CM in heifers occurred before 20 DIM, whereas, half of the total proportion of 2nd and 3rd parity cows with CM was approached around 70 DIM. Before 150 DIM 75% of all first cases of CM had occurred. This shows that cows are more susceptible for cases of CM during the first half of lactation, than during the latter half.
The heritabilities for CM in parity 1, 2 and 3 were all around 3% (Table 1). The genetic correlations between CM in consecutive parities were high (~0.9), but low between parity 1 and 3 (0.6). This implies that CM has not the same genetic background in these parities. The genetic correlations between CM and lactation-average SCS were 0.8 in parity 2 and 3 (Table 2), but somewhat lower in parity 1 (0.6). Even stronger genetic correlations were estimated for SCS in the first half of lactation, but SCS in the latter half of lactation showed weak correlations with CM. From a biological point of view, it is logical that SCS in the first half of lactation is strongest correlated to CM, because 75% of the first cases of CM occur before 150 DIM.

Conclusions: Genetic correlations between CM in consecutive parities are high, but somewhat lower between further aparted parities. Somatic cell score averaged over first half of lactation was found to be strongest genetically correlated with CM.

Acknowledgements: This study is part of the five-year mastitis programme of the Dutch Udder Health Centre and was financially supported by the Dutch Dairy Board.

References:

Table 1: Estimated genetic parameters for clinical mastitis in parity 1, 2 and 3 (CM1, CM2 and CM3, respectively). Heritabilities on diagonal, phenotypic correlations below diagonal and genetic correlations above diagonal, with standard errors as subscripts

<table>
<thead>
<tr>
<th></th>
<th>CM1</th>
<th>CM2</th>
<th>CM3</th>
<th>CM1</th>
<th>CM2</th>
<th>CM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM1</td>
<td>0.03</td>
<td>0.88</td>
<td>0.63</td>
<td>0.64</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>CM2</td>
<td>0.06</td>
<td>0.03</td>
<td>0.91</td>
<td>0.65</td>
<td>0.86</td>
<td>0.88</td>
</tr>
<tr>
<td>CM3</td>
<td>0.05</td>
<td>0.12</td>
<td>0.04</td>
<td>0.50</td>
<td>0.59</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Table 2: Genetic correlations between clinical mastitis in parity 1, 2 and 3 (CM1, CM2 and CM3, respectively), and somatic cell scores averaged from 5 to 335 days, from 5 to 150 days and from 151 to 335 days (SCS5-335, SCS5-150 and SCS151-335, respectively), with standard errors as subscripts

<table>
<thead>
<tr>
<th></th>
<th>CM1</th>
<th>CM2</th>
<th>CM3</th>
<th>CM1</th>
<th>CM2</th>
<th>CM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM1</td>
<td>0.03</td>
<td>0.88</td>
<td>0.63</td>
<td>0.64</td>
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<tr>
<td>CM2</td>
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<tr>
<td>CM3</td>
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<td>0.12</td>
<td>0.04</td>
<td>0.50</td>
<td>0.59</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Figure 1: The proportion of cows with at least one case of clinical mastitis during parity 1, 2 or 3 (CM1, CM2 and CM3, respectively) per day in milk.
SESSION 7: CONTROL OF (HEIFER) MASTITIS III - NUTRITION

029 Control of Heifer Mastitis by Nutrition

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Introduction: While there are many important risk factors that prevail in the cause of bovine mastitis, evidence has been established and continues to grow that links nutrition to mastitis in the dairy cow. Factors linked to mastitis in the mature dairy cow often are associated with mastitis in the first lactation cow also, and many studies have looked at both age groups in their data. There also may be risk factors unique to the heifer due to differences in feeding management during the rearing or pre-calving portion of the heifer program. The majority of data summarized in this review is taken from research involving lactating dairy cows of all ages; however, some limited data are available specifically for first lactation cows.

Discussion: The preponderance of data related to interactions of nutrition and bovine mastitis is found in the areas of trace minerals and vitamins. Trace mineral research has primarily studied copper, manganese, zinc, and selenium. The vitamin research related to mastitis in the dairy cow has focused on vitamins E, A, and beta-carotene. Additionally, several studies have identified other nutrition-related risk factors associated with mastitis.

A major component to raising a healthy dairy heifer is a balanced ration with the key nutrients being protein and energy to allow for a fully functional immune system. However, compromising the immune system takes a major deficiency of protein or energy because the immune system is a component of the maintaince requirement of the growing animal. At the onset of lactation and its related stresses, a protein- or energy-deprived heifer may suffer the effects of a depressed immune system in specific situations. However, studies have not directly shown that dairy cows or heifers fed reasonable amounts of protein and energy to support milk production will have depressed immunity from those two major nutrients (NRC, 2001). On the other hand, specific levels of certain vitamins and minerals have been shown to increase immunity by increasing resistance to infection and by decreasing severity of infections when they do occur. In addition, protein, vitamin A, and zinc influence epithelial cell health and can affect the keratin plug as well as the integrity of the smooth muscle sphincter (Sordillo et al., 1997).

Typically there are two ways that micronutrient deficiencies are believed to impact resistance to mastitis in mature cows. The first way is to weaken the primary defense to mastitis or teat tissue integrity by altering the keratin plug or otherwise impairing epithelial cell integrity before bacterial entry into the mammary gland. The second way is to alter mechanisms by which phagocytes move to the infection site or act to annihilate the bacteria post-entry. It is likely that micronutrient mode of action in mastitis resistance is identical in the dairy heifer.

The dairy cow’s requirements for vitamins and minerals are affected by a variety of factors such as age, pregnancy, and production level or for the heifer, rate of growth (NRC, 2001). It has been accepted that for some vitamins and minerals, the amount required by the animal
for optimal immune function is greater than the amount required for growth and reproduction. By the time clinical signs of deficiency are observed, growth, immunity, and fertility likely have already been compromised (NRC, 2001). Research for many years has studied the impact of nutrition on increased susceptibility to infectious disease. As animal husbandry practices attempt to reduce antibiotic use in farm animals, the concern to study nutrition and immune function has increased, and our data base has grown to include information related to several of these nutrients.

**Conclusion:** Nutrition and feeding management have direct and indirect influences on mastitis. Some specific minerals and vitamins are clearly documented in their influence, while the management of feeding systems is less clear as to how it influences mastitis. Continued research using field data followed by controlled studies is needed in the future to further define the role of nutrition in animal health and in affecting specific mastitis organisms.

**References:**


Feeding in the period around parturition and associations with subclinical and clinical mastitis of primiparous cows in early lactation

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Introduction: In the period around calving, the primiparous cow experiences a lot of physiological changes preparing her for calving and the onset of lactation for the first time while still growing. To cope with all these physiological demands the feeding regime around parturition is important as nutrient deficiency can be associated with increased susceptibility to infectious diseases like mastitis due to immune suppression. For example, cows with hyperketonemia have been shown to have an impaired udder defence due to factors such as reduced phagocytic and bactericidal capacity of neutrophils. In the present study, we wanted to investigate possible associations between feeding around calving and the incidence of subclinical and clinical mastitis of primiparous cows in early lactation.

Materials and Methods: Information on feed and feeding one month before, at, and one month after calving was collected from 72 Swedish large (≥80 cows), high producing, low somatic cell counts (SCC) dairy herds housed in free stall. The study period was from October 2005 to March 2006. Samples of grass silage and grain were taken for analyses of hygienic quality. Data on number of primiparous cows veterinary treated for clinical mastitis (VTCM), breed, age at calving, and SCC, milk yield, percentage of milk-fat and milk-protein, and concentration of milk-urea at first test-milking was collected from the Swedish official milk recording scheme for the 1,189 primiparous cows calving during the study period. Clinical mastitis (CM) was assessed by the number of primiparous cows that had VTCM in the period -10 to 60 days after parturition and subclinical mastitis by number of primiparous cows with a SCC ≥200,000 cells/ml milk at first test-milking. Poisson regression analysis was used to study risk factors at herd level. Associations between the dependent variable and each of the potential risk factors were first screened in univariable Poisson or linear regression analysis. Variables with a P-value ≤0.20, provided that there was no collinearity (r<0.70) between variables, were then considered for further analysis. Only variables with P-values ≤0.05 remained in the final models.

Results: In total, 76 primiparous cows (6.3%; range 0-5 cases/herd) from 42 herds were VTCM in the period -10 to 60 days postpartum. Most cases (47 of 76 cases) occurred during the first week postpartum (day 0 to 7). The geometric mean SCC at first-test milking (occurring on average 20 days postpartum) was 64,300 cells/ml (50% central range: 29,000 – 118,000 cells/ml), and 175 primiparous cows (14.7%; range 0-14 primiparous cows/herd) from 58 herds had a SCC ≥200,000 cells/ml milk at first test-milking. Poisson regression analysis was used to study risk factors at herd level. Associations between the dependent variable and each of the potential risk factors were first screened in univariable Poisson or linear regression analysis. Variables with a P-value ≤0.20, provided that there was no collinearity (r<0.70) between variables, were then considered for further analysis. Only variables with P-values ≤0.05 remained in the final models.
primiparous cows were adapted to the milk cow feeding ration starting ≥3 weeks before calving. Percentage of roughage in the ration given at one month before, at and at one month after calving was 83%, 61%, and 42%, respectively. Corn-silage was used in 11% of the herds at one month before calving and in 22% at calving and onwards. Usage of sugar-beet pulp in the herds was 18% and 44% at one month before and after calving respectively. The most common combination of concentrates to give in all periods was barley, wheat and some commercial industrial processed concentrate. Mean kg of concentrates given to primiparous cows at one month before, at and at one month after calving was 1.8 kg, 5.7 kg, and 12.8 kg, respectively. In 92-97% of the herds minerals were given, and vitamins (mostly vitamins A, D, and E in combination or vitamin E solely) were also given in 15-17% of these, at one month before, at, and one month after calving. Only 5 variables remained with $P$-values ≤0.05 in the final models (Table 1). Feeding factors significantly associated with increasing number of VTCM were to feed less than 13.7 kg of concentrate at one month after calving, to feed sugar-beet pulp at calving and onwards, and not to feed silage from the same batch or silo given before calving as after calving. To feed sugar-beet pulp at one month before calving, as well as to feed corn-silage at calving and onwards was associated with an increasing number of primiparous cows with a SCC ≥200,000 cells/ml at first-test milking.

**Conclusion:** Several feeding factors were found to have a negative association with the udder health of primiparous cows in early lactation. However, these associations need to be investigated further to clarify their impact on udder health, and studies with more detailed information on feeding are advocated.

**References:**

**Table 1:** Final models of the multivariable Poisson-regression analyses of feeding factors significantly ($P$≤0.05) associated with number of primiparous cows veterinary treated for clinical mastitis (VTCM) in the period – 10 days to 60 days postpartum or with number of primiparous cows with a SCC ≥200,000 cells/ml at first test-milking in 72 Swedish dairy herds.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>VTCM</th>
<th>SCC ≥200,000 cells/ml</th>
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<tbody>
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<td>Amount of concentrates given one month after calving</td>
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<td>23</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>2: 12.1-13.7 kg</td>
<td>24</td>
<td>2.28</td>
</tr>
<tr>
<td></td>
<td>3: &gt;12.1 kg</td>
<td>23</td>
<td>2.24</td>
</tr>
<tr>
<td>Beet-pulp is given at one month before calving</td>
<td>1: No</td>
<td>-</td>
<td>59 Ref.</td>
</tr>
<tr>
<td></td>
<td>2: Yes</td>
<td>-</td>
<td>13 1.46</td>
</tr>
<tr>
<td>Beet-pulp is given at calving and onwards</td>
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<td>40</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>2: Yes</td>
<td>30</td>
<td>1.67</td>
</tr>
<tr>
<td>Corn-silage is given at calving and onward</td>
<td>1: No</td>
<td>-</td>
<td>56 Ref.</td>
</tr>
<tr>
<td></td>
<td>2: Yes</td>
<td>-</td>
<td>16 1.43</td>
</tr>
<tr>
<td>Silage from the same batch or silo is given one month before calving and after calving</td>
<td>1: Yes</td>
<td>35</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>2: No</td>
<td>35</td>
<td>1.75</td>
</tr>
</tbody>
</table>

$^1$IRR = incidence rate ratio
SESSION 8: CONTROL OF (HEIFER) MASTITIS IV - MANAGEMENT

031 Management approaches to controlling heifer mastitis

Scott McDougall*, Fiona M. Anniss, Laura Haakma, Katrina I. Parker and Chris W.R. Compton
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* Corresponding author: smcdoug@ahc.co.nz

Introduction: Dairy heifers have a high prevalence of intramammary infection (IMI) prepartum as well as a high peripartum mastitis incidence rate. The epidemiology of mastitis in heifers is different from that of mature cows; hence novel control approaches are required for heifers. Additionally, with increasing focus on reducing antimicrobial use, alternatives to the proven pre partum antibiotic treatment strategies are required. Such strategies may include improved hygiene peripartum, application of teat antiseptics precalving, fly control, separate management of heifers from cows, not feeding colostrum to calves, pre calving milking, modification of diets, use of ionophores, internal and external teat sealants and vaccination.

Material and Methods: Studies were undertaken to assess the effect of internal and external teat sealants, prepartum parenteral antibiotic treatment and prepartum ionophore bolus treatment on the peripartum prevalence and incidence of subclinical and clinical mastitis in dairy heifers. In the first study, 1067 heifers were randomly assigned at heifer-level to one of four treatment groups (no treatment; three intramuscular injections of 5g of tylosin base at 24 h intervals; infusion of an internal teat sealant into all four quarters; three intramuscular injections of 5g of tylosin base and infusion of teat sealant into all four quarters). Mammary gland secretion samples were collected from each quarter before treatment and within five days after calving, and from glands diagnosed with clinical mastitis. In study 2, heifers were randomly assigned within herd to be treated with a slow release intraruminal device containing 32 g of monensin (n = 383) or left as untreated controls (n = 389) approximately 30 days before the start of the spring calving period, or to have a latex external teat sealant applied (n = 206) when calving was impending or be left as untreated controls (n = 205). Mammary gland secretion samples were collected from each quarter before treatment and within five days after calving, and from glands diagnosed with clinical mastitis. For both studies, multivariable analytical techniques were used to assess the effect of treatment prevalence of post-calving IMI infection and incidence of clinical mastitis at gland level.

Results: Study 1. Neither the infusion of a teat sealant (254/2084 (12.2%) vs. 257/2080 (12.4%), RR = 0.99, p = 0.9) nor the administration of tylosin (256/2100 (12.2%) vs. 255/2064 (12.4%), RR = 0.99, p = 0.87) altered the proportion of previously infected quarters curing postpartum. Infusion of the teat sealant reduced the risk of a new IMI with any pathogen prepartum (RR = 0.34, (95%CI 0.26-0.43), p < 0.001), reduced the risk of new infection with Streptococcus uberis prepartum (50/2080 (9.0%) vs. 187/2084 (2.4%), RR = 0.31, p < 0.001), reduced the prevalence of postpartum IMI (154/2080 (7.4%) vs. 458/2084 (22.0%), RR = 0.35, p < 0.001) and reduced the incidence of clinical mastitis from which a pathogen was isolated (34/2080 (1.6%) vs. 130/2084 (6.2%), RR = 0.30, p < 0.001). Parenteral antibiotic treatment had no effect on any of these outcomes (all p > 0.2). Study 2: Monensin treatment did not affect the prevalence of postpartum IMI. The external teat sealant reduced the gland level prevalence of IMI (12.1% (SE 1.7) vs. 16.5% (SE 2.1), p = 0.05) and reduced the prevalence of ‘major pathogen’ IMI (3.8% (SE 0.8) vs. 6.0% (SE 1.1) p = 0.05) compared to control heifers. Neither monensin treatment (60/383 (15.7%) vs. 50/389 (12.8%), p = 0.47) nor external teat sealant (22/206 (10.7%) vs. 25/205(12.2%), p = 0.71) reduced the cumulative incidence of clinical mastitis at heifer level or affected milk production or somatic cell count.
Discussion and conclusions: Use of an internal teat canal sealant in heifers reduced the postpartum IMI prevalence and the incidence of clinical mastitis by decreasing the incidence of new infections during the peripartum period. This study provides the first data that internal teat sealants are effective in heifers when applied prepartum. Other studies have demonstrated that internal teat sealants are effective in reducing the incidence of new infections over the non-lactating period and reducing prevalence of IMI and incidence of clinical mastitis postpartum in adult cattle. With appropriate hygiene at the time of application, the internal teat sealant offers a practical and cost effective tool to reduce subclinical and clinical mastitis in heifers. Parenteral antibiotic therapy proved ineffective in reducing the prevalence or incidence of mastitis in heifers. Whether this was due to pharmacokinetic considerations, antimicrobial resistance or re-infection after therapy remains to be ascertained.

Application of an external teat sealant, but not monensin, resulted in a lower prevalence of any, or a major pathogen, IMI postpartum. Neither treatment affected the incidence of clinical mastitis or milk production or SCC. The internal teat sealant appears more efficacious than the external teat sealant, and has the practical advantage of only needing to be applied once pre-partum. In contrast, the external teat sealant required re-application twice weekly. Despite earlier studies demonstrating that ionophores reduce the prevalence or incidence of mastitis in mixed age cows, ionophore treatment of heifers had no effect on mastitis in the current study. The BOH and NEFA concentrations were lower in monensin treated heifers (data not shown), known risk factors for mastitis and reduced PMN function. However, any benefit of reduced risk of ketosis may have been offset by the higher body condition score of the ionophore treated heifers (data not shown), which may be a risk factor for mastitis.

References:
Heifers teat sprayed in the dry period have reduced *Streptococcus uberis* teat-end contamination and less *Streptococcus uberis* intramammary infections at calving

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**Introduction:** Heifers managed under pastoral conditions are at risk from *Streptococcus uberis* mastitis infections at calving. Researchers agree that the bacterium must first pass through the teat canal to cause an infection. This infers that management options aimed at reducing bacterial loading on teats should hypothetically minimize teat-end exposure to the pathogen and consequently the probability of the animal developing an intramammary infection at calving.

**Materials and Methods:** Experiments were conducted in a research herd (n=54) and five commercial herds (n=343) to a) identify and enumerate *S. uberis* on teat-ends of heifers prior to calving, at calving and two weeks after calving; b) understand the effect of teat-spraying in the dry period on the incidence of mastitis at calving by *S. uberis* or other pathogens. In both studies, heifers were randomly assigned to ‘treated’ or ‘control’ groups. Those on the ‘treated’ group were teat-sprayed once, three times a week (Monday, Wednesday and Friday) with a commercial iodine-based teat sanitizer, starting at three weeks prior to calving and ending at calving day. For both groups, cases of clinical mastitis diagnosed the week after calving were sampled and submitted for bacteriological analysis.

**Results:** Swabbing of research herd animals showed that teat-ends had a pre-existing contamination with *S. uberis*, averaging 610 colony-forming units per swab (cfu/swab) (P=0.78). Prior to calving, *S. uberis* counts on teat-ends were 560 cfu/swab for the ‘treated’ group and 1775 cfu/swab for the ‘control’ group (P = 0.06). Two weeks after calving, teat-end contamination was similar between both groups, at 30 cfu/swab (P = 0.52). The incidence of clinical mastitis post-calving was similar between both treatments (treated = 12.8% heifers vs. control = 11.4% heifers, p = 0.960). However, the incidence of *S. uberis* clinical mastitis was 50% lower in the ‘treated’ (3.6% heifers) vs. ‘control’ group (7.4%) (P = 0.085; Table 1).

**Conclusion:** This study showed that teat-spraying heifers three weeks prior to calving significantly decreased *S. uberis* numbers on teat-ends, also resulting in a marked decrease of clinical mastitis caused by the same pathogen post-calving. It is concluded that teat-spraying in the dry period can be incorporated into the dairy operation for the management of heifer *S. uberis* mastitis in the transition period.

**References:**


**Table 1:** Incidence of clinical mastitis of heifers ‘sprayed’ or ‘not sprayed’ with an iodine-based teat sanitizer in the late dry period.

<table>
<thead>
<tr>
<th></th>
<th>CM cases (CM heifers)/100 heifers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SU (P = 0.085)</td>
</tr>
<tr>
<td>Treated</td>
<td>195</td>
</tr>
<tr>
<td>Control</td>
<td>202</td>
</tr>
<tr>
<td>Total</td>
<td>397</td>
</tr>
</tbody>
</table>
033 Efficacy of vaccination against staphylococci in heifers: A review and new data

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Introduction
Infection of the heifer mammary gland with common mastitis pathogens, particularly staphylococci, prior to calving is well documented. Attempts to eliminate pre-partum infections in heifers have focused primarily on intramammary antibiotic therapy shortly before or at the time of calving. While periparturient intramammary antibiotic therapy has been shown efficacious at reducing intramammary infection (IMI) rates after calving, particularly coagulase negative staphylococcal (CNS) IMIs, economic benefit following such treatment has not been uniformly demonstrated.

A common practice in the control of many infectious diseases is vaccination. Very few studies have evaluated vaccination of heifers to prevent intramammary infections either before or after calving. Commercially available R-mutant Gram-negative mastitis bacterins are widely used on dairy farms to prevent clinical mastitis in the immediate post-partum period. However, research specifically focused on the efficacy of these vaccines in the heifer is lacking. Furthermore, heifer mammary glands are most commonly infected with staphylococcal organisms around the time of calving. There are a few studies evaluating a commercially available Staphylococcus aureus mastitis bacterin (Lysigin, BIVI, St. Joseph, MO, USA) in heifers. We recently compared the efficacy of commercially available Lysigin with two experimental Lysigin formulations and unvaccinated controls in heifers. Heifers were vaccinated twice, 28 days apart in late gestation with either a 3-isolate experimental bacterin (Group I), a 5-isolate experimental bacterin (Group II), or commercially available Lysigin (Group III). All groups (vaccinates and controls) were challenged with a heterologous strain of S. aureus by intramammary infusion on days 6, 7, and 8 of lactation. All cattle became infected with S. aureus after challenge. While 3 cattle in Group I and 1 cow in Group III cleared their S. aureus IMI by the end of the study, there were no differences in S. aureus clearance rates between groups (P ≥ 0.214). Cattle vaccinated with commercially available Lysigin had a lower mean duration of clinical mastitis and lower total mastitis score post-challenge than controls (P = 0.045 and P = 0.046, respectively). Overall, there was no evidence that any of the vaccinated groups had a lower mean somatic cell count (SCC) than control (P ≥ 0.148), and no evidence that vaccinates had greater milk yield than controls post-challenge (P = 0.617). While heifers vaccinated with commercially available Lysigin had higher mean serum IgG1 and IgG2 S:P ratios than controls against S. aureus strains of polysaccharide serotypes 5, 8, and 336 (P ≤ 0.023), milk undifferentiated IgG S:P ratios were only different from control against strains of serotype 8 and 336 (P ≤ 0.030) and no there were no differences between groups for milk IgG1, IgG2, and IgM (P > 0.05). These data suggest that there may be insufficient vaccine-induced IgG2 in milk to opsonise S. aureus and facilitate clearance of the organism. In contrast to our work, Nickerson and co-workers vaccinated heifers with commercially available Lysigin at 6-months of age followed by a booster 2-weeks later and subsequent booster vaccinations every 6-months until calving. Vaccinates had a 45% reduction in both new S. aureus IMI during pregnancy and new S. aureus IMI at calving relative to controls. In addition, vaccinates had a 30% reduction in new CNS IMIs which became chronic and a 31% reduction in new CNS IMI at calving relative to controls providing evidence that Lysigin may be of use in reducing staphylococcal mastitis in periparturient heifers.

In order to further examine the efficacy of Lysigin vaccine against S. aureus and CNS, specifically, under field conditions we recently performed a field trial using a lactating dairy
cow model. The objectives of the study were to evaluate the efficacy of the bacterin in protecting against staphylococcal mastitis (S. aureus and CNS), effect of vaccination on milk SCC, and evaluate the milk antibody isotype response to vaccination when used according to label in lactating cattle.

**Materials and Methods**

Ninety Holstein-Friesian dairy cattle free of S. aureus IMI were systematically assigned to vaccinated (n = 44) or control (n = 46) groups. Vaccinates received two 5-ml doses of Lysigin 14-days apart. Composite milk samples were collected on days 0, 14, 28, 49, and 70 for IgA, IgG1, IgG2, and IgM determinations and SCC enumeration. Mammary quarter foremilk samples for bacterial culture were collected prior to each vaccination and approximately monthly thereafter for 6 months.

**Results**

*Staphylococcus aureus* was isolated from eleven quarters (vaccinates, n = 7; controls, n = 4) during the 6-month follow-up period. However, each of the isolations only occurred on one occasion in a given quarter and therefore did not meet the definition of a new S. aureus IMI (2 of 3 samples positive). The proportion of S. aureus isolations did not differ between groups (P = 0.511). Nineteen quarters on vaccinated cattle and 25 quarters on control cattle were infected with a CNS at the start of the study. Eighteen new CNS IMIs developed in 157 at risk quarters in the vaccinated group and 20 new CNS IMI developed in 159 at risk quarters in the control group (P = 0.895). Incidence of new CNS IMI was 0.82 new CNS IMI per quarter month for vaccinates and 0.81 new CNS IMI per quarter month for controls. Median time to new CNS IMI was 56 days for both vaccinates and controls (P = 0.944). While controls had a higher mean SCC than vaccinates, mean SCC did not differ between groups (P = 0.116) or sample period (P = 0.711), and no group by time interaction was detected (P = 0.273). Differences in mean milk S:P ratios for IgA, IgG1, IgG2 and IgM were not detected between groups (P = 0.965, P = 0.848, P = 0.817 and P = 0.953, respectively) and no group by time interactions were detected (P = 0.524, P = 0.807, P = 0.313 and P = 0.367, respectively). Differences in mean milk S:P ratios were detected between sample days for IgA, IgG1 and IgM regardless of group (P < 0.001). No differences in mean milk S:P ratios between sample days were detected for IgG2 (P = 0.088).

**Conclusions**

Results of this study suggest that 2-doses of Lysigin administered per label in lactating cattle do not reduce the incidence of new S. aureus or CNS IMI. Additionally, using a modification of the Staphylococcus Aureus Antibody Test Kit (VMRD, Pullman, WA, USA) we could not demonstrate a humoral immune response to Lysigin in milk. These data corroborate our challenge trial data in heifers vaccinated twice in late gestation. In contrast to our work, Nickerson and co-workers study suggests that early vaccination followed by multiple boosters before calving may be a useful adjunct in controlling staphylococcal mastitis in heifers.

**References**

034 Individual and herd factors associated with mastitis and bacterial isolates from quarter milk samples drawn from fresh heifers. With special reference to teat dipping routines around first calving

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Introduction: Teat dipping is one of the most important investments applied in prevention of bovine mastitis, and is an essential part of the five point plan. In the last years also external as well as internal teat sealants, have been applied to prevent mastitis. A Norwegian survey, conducted in 1994/95, revealed that 12 % of the farmers applied teat dipping routinely. Another national survey, based on quarter milk samples, discovered that Staphylococcus aureus could be isolated from 22.2 % of the dairy cows (Østerås et al, 2006). It was thus of interest to see if routine application of iodine teat dipping or an external teat sealant could reduce the occurrence of Staphylococcus aureus from quarter milk samples and how teat dipping would impact the bacterial flora in general. A randomized field trial, at herd level, was constructed to evaluate these effects. The herds were enrolled during a two-year period. The main results are published by Whist et al. (2007). The present paper presents how the teat dipping routines were associated with bacterial isolates from quarter milk samples drawn around calving from first lactation cows (heifers).

Materials and Methods: A total of 178 herds were enrolled. The herd were randomly assigned as control (n=71), iodine teat dipping (n=52) or external teat sealant (DryFlex®) (n=55). Altogether 12,872 quarter milk samples from 3,218 heifers (1,347 controls, 930 iodine teat dipped and 941 external teat sealant) were drawn. All heifers were sampled around calving at 6 days in milk. If the heifers experienced a clinical mastitis these were also sampled. The average sample time was 7.7 ± 5.4 DIM. More than half of the samples were taken between 5 and 8 DIM. Heifers sampled after 30 days in milk were excluded. The quarters were characterized as having clinical signs or visible inflammatory changes in the milk or not (1, 0). The bacteria isolated from the quarter samples were classified as having no isolates (1, 0), Staphylococcus aureus (1, 0), Streptococcus dysgalactiae (1, 0), coagulase negative staphylococci (1, 0), Streptococcus uberis (1, 0) or Escherichia coli or coliforms (1, 0). For each of these outcomes a separate regression model was constructed using SAS 9.1 (Cary, NC). Alternative logistic regression was performed with season of calving (winter=Dec, Jan, Feb; spring=Mar, Apr, May; summer=Jun, Jul, Aug and autumn= Sep, Oct, Nov), quarter site (left front, right front, left rear and right rear), teat dipping routine (control, iodine or external sealant) as fixed independent variables and herd and individual heifer as random cluster variables.

Results: Altogether 204 (1.6 %) quarters were assigned as clinically affected. The results from the bacteriological examination were; no isolates in 10.655 (82.8 %) quarters, Staphylococcus aureus isolated from 1,327 (10.3 %) quarters, Streptococcus dysgalactiae isolated from 217 (1.69 %)quarters, CNS isolated from 216 (1.68 %)quarters, Streptococcus uberis isolated from 20 (0.16 %) quarters and coliforms were isolated from 76 (0.59 % ) quarters. The results from the logistic regression models are presented in Table 1.

Conclusion: No significant association between iodine teat dipping or external teat sealant and bacterial isolates could be found except for clinical symptoms and iodine with an OR = 1.6 (0.96 - 2.7) and isolates of coliforms and use of external teat sealant with an OR = 2.9
(1.2 – 7.3). There was significantly less *Streptococcus dysgalactiae* and CNS during the summer and autumn compared to the winter and spring. There was nearly significantly more *Streptococcus uberis* isolates during the summer than the winter with an OR = 9.2 (0.87 – 97). These results are consistent with Østerås et al. (2006). There is a large herd cluster effect for *Streptococcus uberis* and coliforms with an OR = 5.0 (2.6 – 9.9) and 4.9 (3.0 – 8.2), respectively. The rear quarters had generally more frequent isolates than front quarter (OR=1.42). The most predominant isolates on rear quarters were *Staphylococcus aureus* or *Streptococcus dysgalactiae*. Those isolates had a relatively small herd cluster effect, however a large individual cluster effect. This could indicate that breeding for higher resistance could be very important.

**References:**

**Table 1:** Results from the logistic regression models for clinical symptoms (CM) and different bacterial isolates at quarter level at approximately 6 days in milk. Non = No isolate, S.a. = *Staphylococcus aureus*, S.d. = *Streptococcus dysgalactiae*, CNS = coagulase negative staphylococci, S.u. = *Streptococcus uberis*, col. = coliforms. Herd and individual (Heifer) are random effects. RF=Right front, LF=left front, RR=right rear and LR=left rear.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Class</th>
<th>n</th>
<th>CM</th>
<th>Non</th>
<th>S.a.</th>
<th>S.d.</th>
<th>CNS</th>
<th>S.u.</th>
<th>Col.</th>
</tr>
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<td>Intercept</td>
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<td>-6.05</td>
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<td>Season</td>
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<tr>
<td>Winter</td>
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<td>0.03</td>
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<tr>
<td>Teat site</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>3218</td>
<td>-0.05</td>
<td>0.03</td>
<td>-0.07</td>
<td>0.27</td>
<td>-0.19</td>
<td>-0.69</td>
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<tr>
<td></td>
<td>RR</td>
<td>3218</td>
<td>0.24</td>
<td>0.45**</td>
<td>0.44</td>
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<td>0.35***</td>
</tr>
<tr>
<td></td>
<td>LR</td>
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<td>0.33***</td>
<td>0.55***</td>
<td>-0.10</td>
<td>0.15</td>
<td>0.41</td>
<td>0.30***</td>
</tr>
<tr>
<td>Teat dipping</td>
<td>Control</td>
<td>5388</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Iodine</td>
<td>3720</td>
<td>0.48**</td>
<td>-0.06</td>
<td>-0.07</td>
<td>0.20</td>
<td>0.35</td>
<td>-0.46</td>
<td>0.34</td>
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<tr>
<td></td>
<td>Sealant</td>
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<td>0.10</td>
<td>0.13</td>
<td>-0.15</td>
<td>-0.39</td>
<td>1.07**</td>
</tr>
<tr>
<td>Random effects</td>
<td>Herd</td>
<td>158</td>
<td>0.80***</td>
<td>0.08**</td>
<td>0.17***</td>
<td>0.34***</td>
<td>0.47***</td>
<td>1.63***</td>
<td>1.60***</td>
</tr>
<tr>
<td></td>
<td>Heifer</td>
<td>3218</td>
<td>1.58***</td>
<td>1.83***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001, any number 0.05<P<0.10*
*a Random effect of individuals not performed due to few cases in these models.*
Relationships between milk flow traits and udder health in Holstein heifers during the first 120 days of lactation

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Introduction. Milking is a well-known management factor which affects udder health status. The importance of milking machine characteristics and milking procedures in maintaining teat tissue integrity and preventing udder infections is particularly significant in primiparous cows. Indeed, an impairment of teat and udder health status could potentially affect not only the cow performance for the current lactation, but also the next ones. The detailed analysis of milk flow curves during milking by means of electronic milk flow meters provides useful information for assessing milking process. The paper reports the results of a field study aimed to investigate the relationships between milking flow traits and udder health in primiparous cows.

Materials and Methods. A total of 72 primiparous Holstein cows were randomly selected in 6 herds with different milking management and different milking machines. Primiparous cows enrolled in the study were monitored 1 week after calving and monthly thereafter. At each sampling, quarter milk samples (QMS) were aseptically collected. Milk samples were cultured and colonies identified by proper methods according to National Mastitis Council. Somatic cells were counted on a Bentley Somacount 150 (Bentley, USA). Teat tissue status was assessed by teat thickness measurement by the means of a cutimeter, and digital picture of teat apex was taken after milking, transferred on PC and scored by a panel of 3 trained people using reference images. Udder health status was defined on quarter milk sample SCC and bacteriological results for each heifer monthly, as follows: udders with at least one QMS with SCC ≥200,000 cells/ml (either bacteriologically positive or negative) were defined as subclinical; udders with all bacteriologically negative and SCC <100,000 cells/ml QMS were defined as negative; all the other udders were defined as diseased. Teat thickness Δ was calculated as the difference between teat thickness after and before milking [100*{thickness after - thickness before}/thickness before]. Milk flow curves of the whole udder of each heifer were monthly collected with a continuous electronic milk flow meter (Lactocorder, WMB, Switzerland). All the data collected into a 120 d period of lactation were analyzed by ANOVA by using a generalized linear model (GLM procedure; SAS Inst. Inc., Cary, NC) with herd, animal nested in herd, milk yield level and stage of lactation as fixed effects.

Results. On a total of 294 observations, milk yield per milking was on average 13.5 ± 3.8 kg and peak milk flow was 3.3 ± 1.1 kg/min. Milking time was, on average, 7.1 ± 2.4 min. Incline phase was only 0.77 ± 0.43 min but showed wide variability (CV = 56%). Plateau phase had an average duration of 2.8 ± 1.7 min and decline phase showed a very long duration (2.5 ± 1.3 min). Teat apex conditions showed similar scores for each quarter and the average score was low (1.3 ± 0.46).

All the data were grouped based on two different methods: the first one based on monthly udder health status and the second one on overall udder health status of each cow in the first four months of lactation (Table 1). Udders classified monthly as subclinical had significantly lower milk production and, consequently, lower plateau phase and lower average milk flow compared to the others classes. No significant difference in milk flow traits was observed between negative and diseased udders. The results of heifers that had at least one subclinical QMS in any of the first four months of lactation showed significantly higher average and peak milk flows, lower duration of plateau phase, longer duration of incline phase and higher
teat apex score in comparison with the other heifers. Also the ratio between incline phase and plateau phase and the ratio between decline phase and plateau phase were significantly higher in the cows with at least one subclinical QMS in respect to the others, revealing a less favourable milking efficiency. The results in the first four months of lactation (Table 2) showed that heifers with at least one subclinical QMS had a significant increase in peak milk flow while the other heifers had only a slight increase. Teat apex score significantly increased in both groups of heifers as lactation progressed. Teat thickness before milking significantly decreased during lactation only in the group of heifers without any subclinical QMS.

Conclusions. The results obtained in this trial support the presence of a relationship between udder health status and some milk flow traits. Moreover, in primiparous cows changes in teat apex score and teat thickness showed to be associated to the presence of subclinical mastitis during the early months of lactation.

### Table 1: Udder health status and milk flow traits in Holstein heifers (Least-squares means)

<table>
<thead>
<tr>
<th>Item</th>
<th>Monthly udder health status</th>
<th>Overall udder health status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative QMS</td>
<td>Disease QMS</td>
</tr>
<tr>
<td></td>
<td>(n = 142)</td>
<td>(n = 90)</td>
</tr>
<tr>
<td>DIM (d)</td>
<td>65.9a</td>
<td>62.3b</td>
</tr>
<tr>
<td>Milk yield (kg/milking)</td>
<td>14.2a</td>
<td>13.8b</td>
</tr>
<tr>
<td>Time of milk ejection (min)</td>
<td>7.36</td>
<td>7.24</td>
</tr>
<tr>
<td>Average milk flow (kg/min)</td>
<td>2.24b</td>
<td>2.23b</td>
</tr>
<tr>
<td>Peak milk flow (kg/min)</td>
<td>3.37</td>
<td>3.25</td>
</tr>
<tr>
<td>Time of incline phase (min)</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>Time of plateau phase (min)</td>
<td>2.85b</td>
<td>3.00b</td>
</tr>
<tr>
<td>Time of decline phase (min)</td>
<td>2.75</td>
<td>2.48</td>
</tr>
<tr>
<td>Incline:plateau ratio</td>
<td>0.37</td>
<td>0.39</td>
</tr>
<tr>
<td>Decline:plateau ratio</td>
<td>0.98</td>
<td>0.97</td>
</tr>
<tr>
<td>Average teat apex score</td>
<td>1.33</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Means within a row, for monthly and general udder health status separately, with different superscripts differ (P < 0.05)

### Table 2: Udder health status and milk flow traits in the first 4 months of lactation in Holstein heifers (Least-squares means)

<table>
<thead>
<tr>
<th>Lactation month</th>
<th>Heifers without any subclinical QMS</th>
<th>Heifers with at least one subclinical QMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n = 33)</td>
<td>2</td>
</tr>
<tr>
<td>DIM (d)</td>
<td>16.4a</td>
<td>15.0abc</td>
</tr>
<tr>
<td>Milk yield (kg/milking)</td>
<td>13.1a</td>
<td>14.0a</td>
</tr>
<tr>
<td>Time of milk ejection (min)</td>
<td>7.50</td>
<td>7.21</td>
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<tr>
<td>Average milk flow (kg/min)</td>
<td>1.94b</td>
<td>2.26b</td>
</tr>
<tr>
<td>Peak milk flow (kg/min)</td>
<td>2.79</td>
<td>3.29</td>
</tr>
<tr>
<td>Time of incline phase (min)</td>
<td>0.54</td>
<td>0.72</td>
</tr>
<tr>
<td>Time of plateau phase (min)</td>
<td>3.52</td>
<td>2.93</td>
</tr>
<tr>
<td>Time of decline phase (min)</td>
<td>2.28</td>
<td>2.83</td>
</tr>
<tr>
<td>Average teat apex score</td>
<td>1.05ab</td>
<td>1.23abc</td>
</tr>
<tr>
<td>Teat thickness before milking (mm)</td>
<td>13.5a</td>
<td>13.4a</td>
</tr>
<tr>
<td>Teat thickness Δ (%)</td>
<td>-2.7</td>
<td>-1.9</td>
</tr>
</tbody>
</table>

Means within a row, for heifers without or with subclinical QMS separately, with different superscripts differ (P < 0.05)
SESSION: POSTER PRESENTATIONS

P013 Aetiology and risk factors for new intramammary infection in dairy heifers around calving

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Introduction: Culling of persistently infected cows is one of the main actions of control plans against mastitis. However, the expected improvement due to replacement can be reduced if primiparous cows experience subclinical or clinical mastitis at calving or in early lactation. The objective of the study was to describe the bacteria involved and to identify the risk factors for new intramammary infections (IMI) in dairy heifers around calving.

Materials and Methods: 855 heifers from 57 French dairy herds were included. New IMI were determined based on 2 quarter milk samples, implemented 2 days before and 15 days after calving. Fecal contamination led us to restrict the study on 2484 quarters. Cow characteristics and management around calving were described individually. Description of the general management practices was obtained by an interview of each farmer and a farm visit. To assess the risk of occurrence of a new IMI according to the clustering of quarters within heifer and of heifers within herd, a mixed general linear model was defined to identify risk factors.

Results: 9.4% of the quarters had a new IMI with either major pathogens (18%), mainly Streptococcus uberis (Figure 1), or minor pathogens (82%), mainly non-aureus Staphylococci (Figure 2). Four risk factors, related only to cow individual traits, were identified (Table 1): winter calving, dystocia, dirty udder at calving, low milk urea in early lactation. Odds ratios ranged from 1.5 to 3.6.

Discussion: In this study, our approach was original by assessing at the same time quarter, cow and herd risk factors. Calving difficulties and their consequences (lack of hygiene, stress,...) were main determinants of early mastitis. Herd management practices were not associated to mastitis occurrence perhaps because the primiparous cows were exposed to them for a short period.

Conclusion: In herds with a high frequency of subclinical or clinical mastitis in primiparous cows at calving or in early lactation, an improvement may be obtained by a control of the identified risk factors.
Figure 1: Proportion of the main groups of bacteria implied in the intramammary infections with major pathogens

Figure 2: Proportion of the main groups of bacteria implied in the intramammary infections with minor pathogens

Table 1: Frequency, relative risk and confidence interval for risk factors for new intramammary infections around calving (n=2484 quarters)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Frequency</th>
<th>RR</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month of calving</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November-February</td>
<td>50.6</td>
<td>3.60</td>
<td>[2.94; 4.25]</td>
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</tr>
<tr>
<td>March-August</td>
<td>35.6</td>
<td>3.16</td>
<td>[2.4; 3.84]</td>
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</tr>
<tr>
<td>September-october</td>
<td>13.8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calving ease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficult including caesarian section</td>
<td>15.5</td>
<td>1.28</td>
<td>[0.85; 1.71]</td>
<td>0.046</td>
</tr>
<tr>
<td>Normal (assistance of one person)</td>
<td>42.2</td>
<td>1.47</td>
<td>[1.14; 1.79]</td>
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</tr>
<tr>
<td>Easy (no assistance)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Udder cleanliness at calving</td>
<td>dirty</td>
<td>13.0</td>
<td>2.01</td>
<td>[1.52; 2.50]</td>
</tr>
<tr>
<td>clean</td>
<td>50.1</td>
<td>1.59</td>
<td>[1.23; 1.95]</td>
<td></td>
</tr>
<tr>
<td>very clean</td>
<td>36.9</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Milk urea in early lactation</td>
<td>&lt;=250 mg/l</td>
<td>77.6</td>
<td>1.49</td>
<td>[1.10; 1.88]</td>
</tr>
<tr>
<td>&gt;250 mg/l</td>
<td>22.4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \): For each risk factor of new IMI around calving, the class used as reference is underlined.

\( ^b \): Frequency in each class.

\( ^c \): Relative Risk of new IMI around calving [underlined when significantly different from the reference (RR=1.00) \( P < 0.05 \)].

\( ^d \): 95% Confidence Interval of the relative risk of new IMI.
P014 Characterisation of the bovine innate immune response following deliberate intramammary infection with *Streptococcus dysgalactiae*

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**Introduction:** Mastitis is the most economically significant production disease on dairy farms and cost the EU dairy industry more than €1.5 billion in 2005. Current methods of controlling mastitis rely heavily on antibiotics for treatment and prophylaxis. However, antibiotic cure rates for mastitis can be as low as 60%, with lower success rates for subsequent re-infections. Efforts are currently being directed into the development of alternative methods to treat mastitis. In order to fully comprehend how to treat this disease, we must first understand the animal’s immune response to infection with different pathogens. The aim of this study was to investigate the bovine immune response following a deliberate intramammary challenge with *Streptococcus dysgalactiae*.

**Materials and Methods:** Three Friesian cows (T, U and V), in their first or second lactation, with no previous history of mastitis, were selected for this study. One healthy quarter of each animal was infused with a *S. dysgalactiae* preparation (1500 CFU). Blood and total quarter milk samples from infused quarter and control quarter were taken immediately prior to infusion, and 7, 24, 48, 72, 168 and 336h post infusion (PI). Somatic cells were counted and bacteriological enumeration and identification was performed. Gene expression was followed in milk somatic cells and blood throughout the course of infection. RNA was isolated from blood leukocytes and milk somatic cells from both the infused and the control quarter using Tri-Pure reagent. Quality assessed total RNA was DNAse treated and reverse transcribed to create cDNA. Real time PCR was performed using a Lightcycler instrument with Sybr green technology and gene-specific primer pairs for a number of candidate genes (IL-1β, IL-6, IL-8, IL-10, IL-12, TLR2, TLR4, TNF-α, NK-κB, CD14 and CXCR2). Absolute quantification was performed using standard curves generated from known concentrations of cloned gene fragments as template DNA.

**Results:** In Cow V, viable bacteria were first recovered 24 hrs PI, which corresponded to a modest increase in immune gene expression. However peak gene expression was not observed until 168 hrs PI, which coincided with the first notable increase in (SCC). Gene expression of TLR2 and CXCR2 increased 20-fold and 50-fold respectively within this time. Elevated gene expression levels were still observed 2 weeks (336 hrs) PI. Cow U elicited a more rapid response to challenge, with peak gene expression and an elevation in SCC occurring 24 hrs PI. A 200-fold increase in gene copy number of IL-8, IL-12 and CXCR2, a 400 fold increase in IL-1β and a 100-fold increase in TLR2 were all observed within 24 hrs of challenge. This animal continued to display elevated levels of immune gene expression 2 weeks PI. The immune gene expression of Cow T was elevated in the pre infusion sample and did not show any subsequent increase at the sample time points following challenge. However, the milk of this animal was found to contain *Staphylococcus aureus*, which only became detectable following infusion with *S. dysgalactiae*. No elevation was observed in the expression profiles in the blood and the control quarters.

**Conclusion:** To our knowledge, this is the first study into the bovine immune response using real time PCR following a *S. dysgalactiae* challenge. The immune response elicited by these
animals and subsequent antibiotic treatment was not sufficient to clear the pathogen from the cows in this investigation. While a modest increase in gene expression was observed, a greater magnitude of up-regulation would be expected if higher pathogen numbers were used which would potentially trigger a stronger pathogen recognition response in the host. Previous work has shown a difference in the bovine innate immune response when challenged with S. aureus or Escherichia coli. S. dysgalactiae is an intracellular pathogen, allowing it to evade host recognition, thereby hindering the host immune response. This may explain the prolonged nature of infection observed throughout this study. Mechanisms aimed at boosting the immune response to these adapted pathogens may aid in mastitis control.

References:
INTRODUCTION

Intramammary infections (IMI) caused by *S. aureus* are often subclinical and represent a considerable economic loss. Mastitis due to coliforms also generates remarkable losses, particularly in herds with a high sanitary level and with low somatic cell counts.

The results of vaccinating with different potential antigens of *S. aureus* and different adjuvants have been varied so far, both in experimental infection and field trials (5). Regardless of these antigens, exopolysaccharide slime, a complex antigen consisting of proteins and polysaccharides that forms an outer covering on the bacterial capsule and that attaches itself weakly to the latter, has been proven both in vitro and in vivo (1) (4) to be a virulence factor that plays an important role in the adherence of the bacteria to the mammary glandular epithelium. Therefore, the induction of anti-slime antibodies (Ab) could reduce the adherence and consequent colonisation and development of the IMI.

Both in experiments and field trials, it has been proven that vaccinating during the dry period with a mutant strain, *E. coli J5*, reduces the clinical symptoms of cows with mastitis due to *E. coli* (3) and increases the level of Ab against *E. coli J5* (2) (3).

OBJECTIVES

1) To study the seroconversion induced with two doses of a vaccine against *S. aureus* and *E. coli* administered before calving.

2) To assess whether there is a correlation between the level of Ab detected in serum and the level of AC found in individual milk.

MATERIAL AND METHODS

Animals:

Thirty-eight first calving heifers were used, 22 months old, not vaccinated against *E. coli* or *S. aureus* and seronegative to *S. aureus* and *E. coli J5* anti-slime Ab.

Treatments:

Two groups of animals were established. In the first group of 18 animals on which the *S. aureus* anti-slime Ab was tested, 9 heifers were vaccinated with 3 ml of vaccine in accordance with the vaccination plan recommended by the manufacturer (Laboratorios Hipra, S.A.): Vaccination approximately 45 days before the due date of birth (Day 0 of the study) and revaccination 35 days later (10 days after the due date of birth). Within this group of animals, another 9 heifers were inoculated with a placebo (control group) following the same vaccination plan. The placebo used consisted of inoculating the same dose of an emulsion containing the same composition as the vaccine in terms of adjuvant and excipients, while the main active ingredients were substituted with the same amount of PBS solution.

In the second group of animals on which seroconversion to *E. coli J5* was tested, 9 heifers were vaccinated and revaccinated and 11 heifers received the same amount of placebo.

The vaccine used contains inactivated *S. aureus* bacteria (TC5, TC8) which express the exopolysaccharide or slime, and *E. coli J5*, which is a mutated strain lacking the O-polysaccharide chain of the bacterial LPS, and therefore represents the core antigen of the
bacterial LPS, being immunogenic and conserved structurally and antigenically among the Gram negative bacteria.

**ELISA kits:**
Two indirect ELISA kits were used to assess the anti-E. coli J5 and S. aureus slime serological response (2). The G protein was used combined with peroxidase to detect Ab against both pathogens. The reaction catalysed through the peroxidase with the ABTS substrate solution was measured at 405 nm. In bovine serum, the cut-off point was established at an IRPC value = 16.0 for S. aureus anti-slime Ab and IRPC= 34.0 for anti-E. coli J5 antibodies.

**Statistical analysis:**
Descriptive statistics were used (mean and confidence interval) and the Factorial Analysis of Repeated Measurements Model was used to analyse the serological response within the same group and between groups. The correlation between the serological response in blood and in milk was studied via linear regression and the Pearson linear coefficient. Bilateral 95% (two-tailed) was used as the confidence level. The Microsoft Excel 2000 and SPSS 11.5 programs were used for calculations and graphs.

**RESULTS**
The Ab response in serum was assessed on seven different days during the study. The group results are shown below in graph and table form.

**Graph and table 1. S. aureus anti-slime serological response on days post-vaccination**

<table>
<thead>
<tr>
<th>Day post-vaccination</th>
<th>45</th>
<th>0</th>
<th>+14</th>
<th>+35</th>
<th>+49</th>
<th>+69</th>
<th>+73</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated group</td>
<td>5.5</td>
<td>2.9</td>
<td>17.6</td>
<td>13.6</td>
<td>38.1</td>
<td>36.5</td>
<td>35.8</td>
</tr>
<tr>
<td>Non-vaccinated group</td>
<td>7.1</td>
<td>5.6</td>
<td>7.0</td>
<td>7.6</td>
<td>6.4</td>
<td>6.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Arrows indicate days of vaccination. Error bars: correspond to the 95% confidence interval.

**Graph and table 2. Anti-E. coli serological response on days post-vaccination**

<table>
<thead>
<tr>
<th>Day post-vaccination</th>
<th>45</th>
<th>0</th>
<th>+14</th>
<th>+35</th>
<th>+49</th>
<th>+69</th>
<th>+73</th>
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<td>5.6</td>
<td>7.0</td>
<td>7.6</td>
<td>6.4</td>
<td>6.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Table II. Average of group of the response in blood of anti-slime antibodies of anti-E. coli J5 (IRPC/ELISA) at days post-vaccination**
On the day of the last sampling (day 73 for *S. aureus*, day 59 for *E. coli*) the level of Ab in serum and in milk was assessed (pool of 4 quarters) for each individual heifer. Graphs 3 and 4 show the correlation between the *S. aureus* anti-slime and anti-*E. coli* serological response in blood and milk.

**Graph 3.** Correlation between the *S. aureus* anti-slime serological response in blood and milk of vaccinated and non-vaccinated cows on day 73.

- Coefficient of correlation \((r) = +0.948\)
- \(n = 18\) (9 vaccinated + 9 non-vaccinated)
- \(p=2.30 \times 10^{-9}\)

**Graph 4.** Correlation between the anti-*E. coli* J5 response in blood and milk of vaccinated and non-vaccinated cows on day 59.

- Coefficient of correlation \((r) = +0.851\)
- \(n = 20\) (9 vaccinated + 11 non-vaccinated)
- \(p=1.96 \times 10^{-6}\)

**DISCUSSION**

The follow-up of the seroconversion against *S. aureus* slime revealed statistically significant differences \((p<0.05)\) between the vaccinated and non-vaccinated group on day 14 (a single vaccine dose administered), and remained significant from day 49 onwards. After all of the cows in the *S. aureus* group had given birth (day 50 post-vaccination), Ab in individual milk was assessed (day 73 post-vaccination). The Ab response in milk was also significantly higher \((p<0.05)\) in the vaccinated group on day 73. Graph 3 shows a high positive correlation \((r = +0.948)\) and highly significant \((p=2.30 \times 10^{-9})\) of the response to *S. aureus* anti-slime Ab in individual serum and milk for the 18 cows on day 73. Regarding the seroconversion to *E. coli* J5, the differences between the groups were significant in statistical terms on days 14, 49, 55, 59 \((p<0.05)\). Thus, a single vaccine dose was enough to generate a considerable increase of Ab \((d14)\) in relation to the control group, as was the complete two-dose vaccination program \((d49,55,59)\). Once all the cows had
given birth, the serological response in milk on the last day of the trial (d59) was also significantly higher in the vaccinated group (p=0.0311) than in the non-vaccinated group. A high positive (correlation coefficient r = +0.851) and highly significant (p = 1.96x10(-6)) correlation was established between the anti-E. coli J5 serological response in blood and milk samples taken from all of the cows in the E. coli group on day 59 post-vaccination. The results obtained in this study support the hypothesis that serum Ab are transferred through the mucosa to the cistern of every quarter, thus contributing to the generation of local and specific immunity in the udder.

CONCLUSIONS
Vaccination before calving with a combined mastitis vaccine against S. aureus and E. coli J5 induced a higher Ab response in the serum of the two vaccinated groups than in the two control groups that were administered the placebo. There is a positive and highly significant correlation between the level of Ab in serum and in milk, both for S. aureus slime and for E. coli J5.

BIBLIOGRAPHY
P016 Low pathogen load versus high non pathogen infusion in deliberate bovine intramammary challenges.

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Introduction: It is estimated that mastitis affects approximately 30% of dairy cattle in the European Union, with an approximate cost of €150 - €300 per animal. Different causative bacteria lead to large variation in the resulting intramammary infection¹. The reasons for such variation in pathogen related host response are not clear². Therefore, by investigating the bovine response to deliberate challenge with a range of bacteria, mechanisms of host pathogen recognition may be further explored and new targets for treatment may be revealed. This work was undertaken to investigate the bovine response to a deliberate intramammary challenge with a low dose of the mastitis pathogen Streptococcus dysgalactiae and a high dose challenge with an organism generally regarded as safe (GRAS), Lactococcus lactis DPC 3147. This lactococcal strain was chosen as there is evidence of its activity against Gram positive mastitis pathogens in vivo³. The gene expression profiles of selected immune genes were followed over the course of infection in the milk somatic cells.

Materials and Methods: The animals for challenge were chosen based on low somatic cell counts, healthy appearance of udders and the absence of bacterial growth in plated milk samples. The animals chosen for the Streptococcus dysgalactiae challenge included two cows in their first lactation and one in her second. These animals were challenged with 1,500 cfu of this mastitis isolate. For the non pathogen challenge, three cows were infused with 2 x 10^8 cfu/ml of Lactococcus lactis DPC3147. For this challenge, one cow was on her first lactation, the second on her second lactation, neither of which had any previous history of mastitis. The third animal was on her 5th lactation but was included as her somatic cell was very low considering her parity. Full quarter milk samples were collected immediately prior to challenge and at 8h, 24h, 48h and 72h for L. lactis challenge and additionally at 168 and 336h for the S. dysgalactiae challenged animals. The somatic cells were harvested from total quarter milk and the RNA extracted using Trizol. 1 µg of RNA was reverse transcribed to cDNA using the Quantitect Reverse Transcriptase kit (Qiagen). Quantitative gene expression analysis of IL1β, IL8, IL10, TNF α, NFκB, CD14, CXCR2 and the Toll like receptors 2 and 4 was performed on a LightCycler instrument (Roche) using the LightCycler® 480 SYBR Green I Master kit, according to manufacturers instructions.

Results: Following challenge, two of the animals infused with S. dysgalactiae developed clinical mastitis. One of these was in her first lactation and displayed clinical signs within 24h and exhibited maximum gene expression at this time point. The second was in her second lactation and did not present with clotted milk until 192 h (8 days) post infusion while maximum immune gene expression occurred 168h (7days) post infusion. The third animal, a first lactation cow, did not present with S. dysgalactiae mastitis, however Staphylococcus aureus was recovered from her milk from 24h post infusion and at every subsequent timepoint. In addition, the SCC of this animal did not increase in response to the challenge, but remained low through out the experimental period, nor did she develop clinical signs of mastitis. In addition, the expression of immune genes under analysis displayed high levels at time zero and did not increase thereafter. The three animals challenged with L. lactis elicited
signs of udder inflammation 8h post infusion. By 48h post infusion all three animals presented with clotted milk. In the L. Lactis challenged animals the peak time point for all immune gene expression was at, or before 24 h with the heifer displaying a second peak in IL8 and CD14 at 72h post infusion.

**Conclusion:** Deliberate intramammary challenge with a high dose of the non pathogenic organism Lactococcus lactis resulted in udder inflammation and clinical signs of mastitis in the three animals in this investigation. All immune genes under investigation were up regulated within 8h post infusion. The L. lactis induced mastitis was self limiting and no animal required antibiotic intervention. Animals infused with a low dose of S. dysgalactiae were slower to respond even though this strain was isolated from a mastitis case. Once this pathogen had been introduced to the udder it proved difficult to treat with antibiotics. In one first lactation animal viable S. dysgalactiae were still recovered after 7 days of antibiotic treatment while expression of the immune genes had decreased significantly following an initial peak at 24h. One heifer infused with S. dysgalactiae did not succumb to mastitis, however it became apparent that she had an underlying S. aureus infection. It is possible that infusing with S. dysgalactiae mobilised the second pathogen out of the cells allowing detection in the milk. The SCC of this animal was low to begin with and microbiology on the milk was negative. However, there were high levels of immune gene expression, particularly IL8, IL1β, CXCR2 and TLR4 in the milk somatic cells immediately prior to infusion. Therefore SCC in some cases may remain low even though there is underlying infection and immune stimulation which may affect milk quality. Animals infused with higher numbers of a non pathogenic organism displayed a more rapid and pronounced immune response than those challenges with a low level of the pathogen Streptococcus dysgalactiae.

**References:**
Introduction: Once a day (OAD) milking may offer a significant opportunity to improve labour output and reduce costs on dairy farms. An increase in somatic cell count (SCC) with OAD milking has been reported with no difference observed in the level of clinical mastitis infection\(^3\). OAD cows also produce less milk compared to twice a day (TAD) milked cows\(^2\). Teat hyperkeratosis (HK) is a teat condition which results from the normal physiological process of adaptation to milking and changes in teat condition due to milking may reduce the effectiveness of the teat canal against infection\(^5\). Higher yielding cows have higher HK scores and this may be due to longer milking times\(^4\). Hence, the condition of cows teats milked once per day may be improved due to fewer milking occasions. On the other hand, a longer duration of milking on one occasion per day could negatively affect teat condition. However, when this was tested with pluriparous cows milked TAD and OAD differences in teat condition were not observed\(^1\). But significant differences in teat condition due to milking frequency may be apparent with primiparous cows. The objective of the current study was to compare the effect of milking frequency (OAD v TAD) on the udder health (somatic cell count, clinical mastitis, teat condition) and milking characteristics of primiparous cows over a complete lactation.

Materials and Methods: Forty spring calving, primiparous Holstein-Friesian cows were blocked according to expected calving date to either OAD or TAD milking frequency treatments at calving. Cows were milked as one group in a 20-unit, 80-degree side-by-side milking parlour. Pre-milking teat preparation consisted of washing with warm running water and drying with individual paper towels. Clusters were automatically removed when milk flow-rate dropped to 0.2 g/min with a delay time of 20 s. OAD cows were milked at 0730h and TAD cows were milked at 0730 and 1530h. Teats were disinfected post milking. Bulk milk samples (OAD at am, TAD at am + pm) from individual cows were taken fortnightly to measure SCC. Clinical cases of mastitis were recorded and milk samples of infected quarters were taken for microbiological analysis. Milking characteristics including time to milk letdown (s), milk time (s), maximum and average milk flow-rate (kg/min) were recorded for each milking during peak lactation (day 30 to 90) using electronic milk meters. Teat orifices were classified (scale 1 to 5) monthly for HK. Score1 was a normal teat-end orifice and score 5 was classified as severe. Milking characteristic data for the morning milking were transformed using the natural logarithm. The resulting transformed data was normally distributed based on the Kolmogorov-Smirnov test. The data were analysed using mixed models (PROC MIXED; SAS, 2006). Fixed effects included in the model were milking frequency and month of lactation. Cow was included as a repeated effect with a first order autoregressive correlation structure with heterogenous variances assumed among month of lactation within cow. For lactation data, mixed models were also used with milking frequency included as a fixed effect and block was included as a random effect. Least squares means of the transformed data were extracted and back-transformed for ease of interpretation.

Results: There were no differences in milking characteristics for primiparous cows milked either once daily or twice daily during the peak months of lactation (Table 1). Milk yield did not differ between treatments at the morning milking and as a consequence there were no differences observed in milk flow-rates. However, lactation milk yield was significantly higher for TAD (3719kg) compared to OAD (2591kg) cows (P<0.001). There were 2 and 3 new
clinical mastitis infections recorded for OAD and TAD cows, respectively. The pathogens typed in quarter milk samples were Escherichia coli \((n=1)\), Staphylococcus aureus \((n=3)\) with no pathogens detected in one sample. Mean SCC was low for both milking frequencies over the lactation, however TAD (82,000) had significantly lower SCC compared to OAD (142,000) cows \((P<0.001)\). HK scores were numerically higher for OAD compared to TAD and increased as lactation progressed for both treatments (Figure 1).

Conclusion: Milking primiparous cows once daily compared to twice daily did not affect milking characteristics such as milk time or milk flow-rate, teat condition or new infection rate. However SCC increased with OAD milking by 60,000 cells/ml.

References:

Table 1: Daily milking characteristics (day 30 to 90) and lactation milk yield, somatic cell count and teat score for primiparous cows milked once a day (OAD) and twice a day (TAD) (back transformed means are in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>OAD</th>
<th>TAD</th>
<th>s.e</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morning milking data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield (kg/c)</td>
<td>2.53 (12.5)</td>
<td>2.54 (12.7)</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Time to milk let down (s/c)</td>
<td>3.72 (41)</td>
<td>3.85 (47)</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Milk time (s/c)</td>
<td>5.96 (387)</td>
<td>5.92 (372)</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Max flow-rate (kg/min/c)</td>
<td>1.21 (3.4)</td>
<td>1.22 (3.4)</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Average flow-rate (kg/min/c)</td>
<td>0.69 (2.0)</td>
<td>0.063 (2.0)</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Lactation data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation milk yield (kg/c)</td>
<td>2591</td>
<td>3719</td>
<td>183</td>
<td>***</td>
</tr>
<tr>
<td>SCC log(_{10}) (x10(^3) cells/ml/c)</td>
<td>4.95 (142)</td>
<td>4.41 (82)</td>
<td>0.23</td>
<td>***</td>
</tr>
<tr>
<td>Mean cow teat score</td>
<td>9.1</td>
<td>8.3</td>
<td>0.61</td>
<td>NS</td>
</tr>
</tbody>
</table>

Figure 1: Mean hyperkeratosis score for cows milked either twice a day (TAD) or once a day (OAD) over the lactation.
P018 Metabolic Changes in lactating cows during experimentally induced mastitis with endotoxins or Escherichia coli.

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Introduction: The incidence of clinical E. coli mastitis is highest between parturition and peak lactation (4). During E. coli mastitis, large quantities of endotoxins or lipopolysaccharides (LPS) are released during the exponential growth of the bacteria and during their lysis. LPS is a potent inducer of proinflammatory cytokines (5). Changes in circulating hormones and liver enzymes differ between intravenous and intramammary (i.mam.) administration of LPS (2). The purpose of this study was to compare the effects on the changes in the cows’ metabolic profile after i.mam. E. coli or LPS.

Materials and Methods: Twelve East Flemish Red Pied early lactating cows with the milk somatic cell count (SCC) lower than 100.000 cells/ml and negative for major pathogens were used. To allow acclimatization to the new environment, cows were transferred in individual tie-stalls one week before experiment. Half of the cows were infected with 1*10^4 cfu/quarter of E. coli (EC) strain P4:O32, while remaining cows were infused with 500 µg/quarter of LPS from E. coli strain O111:B4. Both challenges were done in front and rear left quarters. Controls concerned diet, milk yield and clinical signs (rectal temperature, heart rate, rumen motility) as previously described (3). At 1 hour after the morning milking (1.5 hours after the morning meal), blood samples were collected once daily on day -4, -1, 0, +1, +2, +3, +6 and +9 from EC and LPS challenges. On d0, blood samples were collected immediately before infections (hour 0) and every two hours till 14th hour after inoculations. Plasma levels of glucose, urea, total cholesterol, haptoglobin, ceruloplasmin, total protein, globulin, albumin, Aspartate transaminase (AST), g glutamyl transpeptidase (GGT), total bilirubin, Ca, Zn, non esterified fatty acid (NEFA), β-hydroxybutyrate acid (BHA), vitamin A and vitamin E were analysed using the methods described by Bionaz et al. (1). Data were subjected to ANOVA using the repeated statement in the MIXED procedure of SAS package (Cary, NC, release 8.0) including in the model cow within treatment, treatment and time from treatment as main factors.

Results: Milk yield, rectal temperature and heart rate changes have been previously showed (3). Both treatments showed a pronounced effect at blood level, which typically appears during inflammatory events: Zn and Ca were the most rapidly reduced (since 6-8 hours) and positive acute phase proteins were augmented in some extent, but with some delay (for haptoglobin it appears after 14-16 h as showed by Hoeben et al., 2000). Nevertheless, some difference emerged comparing the two treatments. Zn and Ca were quickly reduced with LPS since 6th hour after challenge (P<0.001 vs d0), while in EC the marked decrease begun from 10th hour. The almost total recovery was anticipated in LPS, but only for Zn in significant manner (10.3 vs 6.4 mmol/l of EC at 48th hour; P<0.01). Both treatments caused a similar increase and pattern of change of the haptoglobin (3). The ceruloplasmin, another positive acute phase protein, was slightly reduced immediately after both challenges (N.S.) and showed only a weak rise from 6th day after both treatments, significant only in EC (P<0.05).

Among negative phase proteins, synthesized by liver and reduced when a inflammation occurs, we have considered albumins, lipoproteins and the carriers of vitamin A and E (respectively evaluated as total cholesterol, vitamin A and E). Albumin and vitamins A and E
showed a marked (P<0.001) reduction during both treatments. The pattern of diminution of albumin was quicker in LPS vs EC (it started after 4 vs 10 hours), this justify the lower level observed in LPS vs EC (P<0.05) from 4 to 12 hours. Also vitamins A and E were reduced quicker in LPS vs EC, in fact the minimum levels were reached 24 hour after treatment (about -37 and -20% respectively; P<0.001); nevertheless, EC group showed the most marked and persistent reduction (P<0.01 vs d0 till the end of experiment). Total cholesterol (lipoproteins) has showed a relevant change in both groups and very prolonged in EC cows (nadir was observed at 144 hours).

Between metabolic indices, glucose showed the typical biphasic rises (e.g. peaks after 6-8 and 24 hours) with both treatments, but the 1st peak resulted anticipated of about 2 hours in LPS vs EC. NEFA showed a transient increase after treatments (instead of the typical post feeding reduction); again the peak appeared earlier in LPS. On the contrary BHA was reduced and remained lower. Finally, urea also showed a marked rise with maximum at 24 and 48 hours (P<0.05 vs d0) in both the treatments, followed by a progressive decrease afterwards. Creatinine was slightly increased in the first 10-14 hours and till 24 hours in EC, then it was gradually reduced to values lower than basal ones.

Conclusion: The results of this experiment clearly confirm that the effects on metabolism and on liver functions are very similar when mammary gland inflammation is either induced by E. coli mastitis model or LPS infusion. Despite LPS effects appear quicker, shorter and sometimes less pronounced, both treatments cause the usual changes due to pro-inflammatory cytokines: i) increase of positive acute phase proteins and reduction of the negative ones, without apparent liver damage; ii) temporary catabolic condition with lipolysis and proteolysis.

References:
Introduction: Prolactin (PRL) and Growth hormone (GH) play an important role in hormonal regulation of lactation. PRL, released in plasma in high concentrations at calving, acts on differentiation of the mammary gland epithelial cells and contributes to lactogenesis. In ruminants, GH is necessary for the maintenance of lactation and its plasma levels are positively correlated with milk yield (3). The importance of GH, PRL and IGF-1 in the immune system has already been recognized (6). The GH receptor has been identified on polymorphonuclear leukocytes (PMN) in many species. GH augments the differentiation of bone marrow derived PMN and enhances a number of immune responses in humans (1, 4). Our aim was to check the hypothesis that naturally high GH levels could enhance resistance against severity of E. coli mastitis.

Materials and Methods: Twelve healthy early postpartum Holstein Friesian cows were used. Clinical mastitis was induced in all cows infusing 10 ml pyrogen-free 0.9% NaCl with $1 \times 10^4$ cfu E. coli strain P4 into the front and rear left udder quarter, immediately after the morning milking at 8 a.m. Blood samples were collected from the external jugular vein into Vacutainer tubes (Lithium heparin) at: -96, -24, 0, +2, +4, +6, +8, +10, +12, +18, +24, +48, +72, +72, +144 and +216 hours from challenge. The number of leukocytes in whole blood was determined and the immature neutrophils were identified as metamyelocytes and myelocytes and band neutrophil. Plasma was stocked in several aliquots at -20°C for GH, IGF-I and PRL quantification and for glucose and non esterified fatty acids (NEFA), this last one using a method previously described by Bertoni et al. (2). Quarter milk production, rectal temperature, heart rate and rumen motility were registered at the times of blood sampling. Data were analyzed with a two way ANOVA, including group and time in the model, using the statistical analysis program package Statistix (Analytical Software, Tallahassee, FL, USA). Challenged cows were scored on severity and retrospectively divided into 2 groups: moderate (M, 9 cows) and severe (S, 3 cows) diseased cows. The qualifying criteria for severity was a milk production higher (M) or lower (S) than 50% of the pre-challenge milk production at 2 days after challenge, according to Heyneman et al. (5). Differences between S and M groups were evaluated using the Kruskal-Wallis test, because variances were not equal in the two groups according to a Bartlett’s test.

Results: According to the severity estimation criteria, milk production was recovered in M cows at day +3 post challenge, but remained low in S cows until +9 days after challenge. Rectal temperature increased monophasically in M and biphasically in S cows with peaks of 40.5°C at +10 h (S and M cows) and 39°C at +24 h (only in S cows) after challenge. Before challenge, plasma NEFA levels were slightly higher in M (0.23±0.04 mmol/l) than in S cows (0.14±0.06), but during E. coli challenge NEFA grew more in M than in S cows (P<0.001). Plasma glucose levels were similar in M and S cows (about 3.5 mmol/l) before and after challenge. Before challenge, plasma GH levels were consistently higher in M than
in S cows (P<0.001), while plasma IGF-I and PRL levels were not significantly different between the two groups. Whereas the general trend of plasma GH was similar in M and S cows, average values remained higher in M cows throughout the experiment (P<0.001). Fluctuation of plasma IGF-I levels were observed in both groups, but there were no significant differences between the groups. No changes of plasma PRL levels were observed in M cows, while in S cows, their levels increased biphasically with significant (P<0.05) peaks at +2 and +10 h post challenge. The last PRL peak coincided with the nadir of plasma NEFA and the increment phase of fever. Before challenge, blood PMN numbers were significantly higher in M than in S cows (P<0.01) which is consistent with previous observations. Between +8 and +24 h after challenge, blood PMN numbers were significantly decreased in both M and S cows, this being significantly more pronounced in S cows. There were no immature neutrophils in the blood of all cows before challenge. The percent mature PMN in blood decreased rapidly in both groups from +8 h post challenge to reach minimal values at +12 h post challenge.

**Conclusions:** The higher values of GH observed in M group can be due to many causes: genetic background, more pronounced energy balance (higher NEFA), stress conditions etc.; however, of great interest are these higher values and the higher GH response during challenge in M cows in conjunction with a decreased number and maturity of blood PMN. Impaired granulopoiesis could be related with severe clinical response against intramammary *E. coli* infections and could possibly be related with decreased GH plasma levels (observed in S group). In conclusion, we found a decreased number and maturity of PMN in S cows before and during induced *E. coli* mastitis. Plasma GH levels were 2.5 times lower in S than in M cows, whereas IGF-I levels were similar. In addition, we report here for the first time a biphasic PRL release in S cows during induced *E. coli* mastitis, whereas in M cows only a monophasic PRL release was observed; its meaning, nevertheless, does not appear so clear.

**References:**

**Table 1:** Average values of some parameters in plasma of early post partum cows before (B) and from 0 to 216 h (A) intramammary *E. coli* challenge. *P<0.05; **P<0.01; ***P<0.001

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mmol/l)</th>
<th>NEFA (mmol/l)</th>
<th>GH (ng/ml)</th>
<th>IGF-I (ng/ml)</th>
<th>PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>M</td>
<td>3.57</td>
<td>3.77</td>
<td>0.255</td>
<td>0.256</td>
<td>12.1</td>
</tr>
<tr>
<td>S</td>
<td>3.49</td>
<td>3.67</td>
<td>0.143</td>
<td>0.147</td>
<td>5.7</td>
</tr>
</tbody>
</table>
**P020 Effect of prepartum intramammary infusion of Albadry Plus on the rate of postpartum Staphylococcus aureus infections of the heifer's mammary glands**

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**Introduction:** Staphylococcus aureus is a very important cause of mastitis in the cows and heifers. Occasionally this bacteria causes the gangrenous mastitis in the heifers at pre or postpartum periods (Author observations). There are many researches concerning the cow’s mastitis (1,2) but a little knowledge is about the heifer mastitis caused by Staph aureus. The purpose of this research was to determine of the Albadry Plus efficacy in the mammary glands of heifers for elimination of the Staph aureus bacteria at the prepartum period.

**Materials and methods:** A total number of 60 heifers with the positive intramammary culture of Staph aureus, were selected in the farms of Tabriz (North-west of Iran), one month prior to predicted parturition time, the heifers were divided into two groups of A and B (n=30). In the group of A, a tube of Alba dry plus (10ml of a syringe contains: Novobiocin (Na) 300mg, penicillin Benzyl procaine 200000 IU) and Aluminum Monostearate until 10ml, produced by Pfizer Company) was infused into each quarters. In the group of B, Albadry were not infused into quarters of heifers. After parturition, milk of all quarters of heifers in the two groups, were cultured again.

**Results:** In the group of A, Staph aureus were isolated from 7 heifers (23.4%), and 23 heifers (79.6%), found negative. In the group of B, 28 heifers (93.3%) were found positive to Staph aureus and from 2 heifers (6.7%) this bacteria were not isolated. Statistical differences were found between two groups (p≤0.05).

**Conclusion:** These results strongly indicated that intramammary infusion of Albadry Plus, one month before of parturition time could be decline the Staph aureus bacteria from the mammary glands of heifers.

**References:**

**Table 1:** Rates of Staphylococcus aureus infections in the heifers after parturition

<table>
<thead>
<tr>
<th>Group</th>
<th>Staph (-)</th>
<th>Staph (+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23 (76.6%)</td>
<td>7 (23.4%)</td>
<td>30(100%)</td>
</tr>
<tr>
<td>Treated with the Albadry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2 (6.7%)</td>
<td>28 (93.3%)</td>
<td>30(100%)</td>
</tr>
</tbody>
</table>
**P021 Auction stress – predisposing factor of mastitis in heifers?**

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**Introduction:** Heifers in high pregnancy have an increased risk that the lorry transport from the farm to the auction localization will evoke problems (traumata, infections, mastitis, calving problems, etc.)\(^2\)\(^3\). Moreover, it is very difficult to determine the udder health status precisely with common cytological and bacteriological tests for milk/secret analysis from heifers just prior to calving. Both aspects resulted in the decision by the majority of German Cattle Breeding Associations to sell at auctions only heifers after calving at their home farm about 1 month later (with or without their calves) as lactating heifers. Very little information is available to what extent the “auction stress” will influence the udder health status in lactating heifers about 1 month after calving. The international literature reported throughout the last years a plenty of papers on the infection status of the mammary glands in freshly calved heifers, yet only to very limited extent information on udder health in heifers after one month after calving has been published. Therefore, this study details the udder health at quarter level of heifers at their home farm and the trend in udder health after the transport from the farm to the auction hall and about six hours later (after the auction procedure).

**Materials and Methods:** A total of 142 German Holstein heifers, lactating since about one month, selected from 25 different dairy farms with an average milk yield of about 10,000 kg/ cow and year were included. All heifers were clinically healthy. The 568 udder quarters were sampled (quarter foremilk samples: QFS) at three occasions: OC-1: The day before auction; before afternoon milking; OC-2: day of auction; after transport and arrival at the auction hall; OC-3: about 6 hours after OC-2, when the auction procedure had finished. Machine milking was performed just after OC-1 and after OC-3. All QFS were used for cytobacteriological examination and for measuring NAGase activity and electrical conductivity. Counting of somatic cells was done by Fossomatic® (precision: Cv < 5%) and bacteriological examination was performed according to National Mastitis Council recommendation. N-acetyl-β-glucosaminidase (NAGase) was measured by the means of a fluorometer (Labsystems®). The determination of the electrical conductivity (EC) was done by the Mastitron® (Fa. Milku). Udder health was categorized based on DVG definition with a cell count threshold of 100,000 cells/ml (Normal secretion (NS); latent infection (LI), unspecific mastitis (UM) and mastitis (M)).

The statistical analysis was performed by using SAS-procedures (PROC UNIVARIATE; PROC GLM).

**Results:** The comparison of the distribution (%) of the four udder health categories in relation to the sampling occasion (OC-1. At the home farm; OC-2: One day later after arrival at the auction hall; OC-3: About six hours after CO-2) is detailed in Table 1. Overall, 386 quarter (68%) indicated NS. Infections could be identified in 160 quarters (28,12%) as LI or M. The data are comparable at all three samplings. Throughout all three samplings, 35 quarters (9.6%) out of the NS-group at CO-1 (n = 365) changed to the category LI (n = 32) or to UM (n = 3). Table 2 compares the main types of pathogens causing latent infections (LI) or mastitis (M). Coagulase-negative *Staphylococcus* species (CNS) accounted for 67,2 % of bacteria isolated, followed by 19,2 % for *coryneform bacteria* and 9,7 % for *S. aureus*. The group other pathogens comprised 4%. The predominant occurrence of CNS has also been reported in other studies\(^4\). The presentation of cyto-biochemical mean values in Table 3 reflects the physiological range for all parameters throughout the study. Therefore, the significant differences between the means for CO-1 and the other two samplings are not of practical importance.
Conclusion: In contrast to the very common assumption that stress may induce new intramammary infections in bovines, the results of this study did not confirm this view. All measured parameters (udder health categorizations, mastitis causing pathogens) did not show significant differences between before (OC-1) or after (OC-2) the transport of the heifers to the auction and also at the sampling six hours later (CO-3). The main conclusion consists in the statement that healthy quarters will be not affected by stressors as transport to the auction hall or the auction procedure, so that new infections will occur. Similar data resulted from other stressors applied to lactating cows.

References:
1. DVG (German Veterinary Medical Society) (2002). [Relevant aspects of controlling bovine mastitis as herd problem]. DVG, Gießen, Germany.

Table 1: Comparison of udder health categories distribution (%) at three samplings (OC-1; OC-2 and OC-3; n = 568 quarters)

<table>
<thead>
<tr>
<th>Udder health category</th>
<th>OC-1</th>
<th>OC-2</th>
<th>OC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal secretion</td>
<td>64.61 %</td>
<td>70.07 %</td>
<td>69.54 %</td>
</tr>
<tr>
<td>Latent infection</td>
<td>24.12 %</td>
<td>22.01 %</td>
<td>22.71 %</td>
</tr>
<tr>
<td>Unspecific mastitis</td>
<td>3.70 %</td>
<td>2.46 %</td>
<td>2.30 %</td>
</tr>
<tr>
<td>Mastitis</td>
<td>6.34 %</td>
<td>4.05 %</td>
<td>5.11 %</td>
</tr>
</tbody>
</table>

Table 2: Comparison of mastitis pathogen distribution (%) at three samplings for all pathogens isolated (OC-1; OC-2 and OC-3; n = 568 quarters)

<table>
<thead>
<tr>
<th>Mastitis pathogen</th>
<th>OC-1</th>
<th>OC-2</th>
<th>OC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>83 (62%)</td>
<td>26 (72%)</td>
<td>79 (63%)</td>
</tr>
<tr>
<td>Corynefor.bact.</td>
<td>42 (32%)</td>
<td>5 (14%)</td>
<td>36 (29%)</td>
</tr>
<tr>
<td>S.aureus</td>
<td>8 (4%)</td>
<td>4 (11%)</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (2%)</td>
<td>1 (3%)</td>
<td>6 (5%)</td>
</tr>
</tbody>
</table>

Table 3: Mean values (X±; ± std.) of different cyto-biochemical parameters in QFS related to three samplings (OC-1; OC-2 and OC-3; n = 568 quarters)

<table>
<thead>
<tr>
<th>Parameter (dimension)</th>
<th>OC-1</th>
<th>OC-2</th>
<th>OC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elec. conductivity (mS/cm)</td>
<td>5.67 ± 0.52</td>
<td>5.76 ± 0.46***</td>
<td>5.97 ± 0.53***</td>
</tr>
<tr>
<td>NAGase (mmol<em>ml⁻¹</em>ml⁻¹)</td>
<td>2.09 ± 1.95</td>
<td>1.81 ± 1.46***</td>
<td>1.75 ± 1.31***</td>
</tr>
<tr>
<td>Cell count (log/ml)</td>
<td>4.13 ± 0.62</td>
<td>3.81 ± 0.72***</td>
<td>3.92 ± 0.69***</td>
</tr>
</tbody>
</table>

Significance versus OC-1: *** = p < 0.0001
P022 Effect of organic zinc supplementation on somatic cell count in cow milk

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Introduction
The aim of the study was to investigate the effect of organic zinc source supplementation on somatic cell counts (SCC) in milk of cows in peak lactation.

Material and methods
The study included 50 Holstein cows, with milk yield from 9,260 to 12,526 kg per 305 day lactation. The study was carried out in a herd with a long history of zinc deficiency. Zinc concentration in total mixed ration /TMR/ was 36 mg Zn/kg on dry matter (DM) basis.
Lactating cows were divided into two even groups: Supplemented (S) and Control (C). All the cows received the same diet. The group S received 30 mg organic zinc source (Bioplex Zn) / kg feed on DM basis daily. Mean dietary DM consumption was 22.8 kg in both the groups.
From all the cows, milk samples were collected on a monthly basis, and SCC determined by Fossomatic. In 6 Control cows and 6 Supplemented cows, serum zinc concentrations were measured at the start of the trial, and at three month intervals, using the AAS method. All the cows were monitored for the health status and mastitis occurrence for the whole trial period.

Results
The initial mean somatic cell counts in the Supplemented and Control were 271,440 ±74,914 and 299,640 ±79,964, respectively. In the Supplemented cows, SCC in milk was gradually decreasing to 151,000 ± 48,342 at the end of the trial, whereas the Control showed only a slight decrease in SCC. A decrease in SCC of the Supplemented was significant.
The 6 Supplemented cows under study showed an increase in serum zinc concentrations, too. The initial serum zinc concentrations in the Supplemented and Control were 11.46 ± 1.04 µmol/l and 11.08 ± 1.17 µmol/l, respectively. After 3 months, serum zinc concentration in Supplemented cows was increased to 13.66 ± 0.9 µmol/l, and in Control cows it was 11.14 ± 1.1µmol/l. At the end of trial, serum zinc concentration in the Supplemented group was increased to 15.05 ± 0.98 µmol/l, whereas in the Control it was 11.78 ± 0.59 µmol/l.

Conclusions
The results imply that the elimination of zinc deficiency by organic zinc supplementation has a favourable effect both on somatic cell counts in milk and serum zinc concentrations in dairy cows.

The study was carried out within the project 1G46086 of the Czech Ministry of Agriculture, along with the Alltech company.
P023 Influence of lactation somatic cell count on milk production of dairy heifers in Argentina

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Introduction: The measure of individual somatic cell count (ISCC) is used worldwide as a surveillance tool to trace subclinically infected cows (1, 6). Intramammary infections (IMI) have been recognized as major factors that influence ISCC (5, 7). Several studies have clearly shown the impact of ISCC in heifers on somatic cell counts (2, 4) and milk production (2, 3) over the first lactation. However, this impact has not been evaluated in Argentina. The purpose of this study was to determine the influence of lactation somatic cell count (LSCC) on milk production of dairy heifers in Argentina.

Materials and Methods: From January to December 2006 monthly ISCC and milk production records during the entire lactation were collected from 7,952 heifers in 113 commercial dairy herds (ranged between 70 to 750 milking cows) of Argentina located in Province of Buenos Aires, Santa Fe, Cordoba and Entre Rios. Geometric mean LSCC records ≤100 x 10^3 cells/ml, 101-300 x 10^3 cells/ml, 301-500 x 10^3 cells/ml and 501-1000 x 10^3 cells/ml were allocated to classes 1, 2, 3 and 4, respectively. Differences between LSCC classes on mean Kg 305-day lactation milk production were analyzed by Repeated Measures/General Linear Model.

Results: Table 1 shows the distribution of LSCC classes and influence on milk production in dairy heifers. Geometric mean of LSCC was 64,000 cells/mL, ranging from 5,000 to 3,400,000 cells/mL. Heifers in class 1 produced 150, 425 and 600 kg more during lactation in comparison with classes 2, 3 and 4, respectively. The LSCC effect on milk production showed significant differences between class 1, in comparison with classes 3 and 4. The total loss caused by classes 3 and 4 during lactation in comparison with class 1 was 7.39 and 10.43%, respectively. LSCC pattern in classes 3 and 4, was characterized by persistent elevation of ISCC throughout lactation.

Conclusions: LSCC above 300,000 cell/mL in heifers impact on lactation production. Increased LSCC was associated with a reduced value of productivity in heifers. Decreased lactational production was associated with permanently elevated ISCC throughout lactation. The main detrimental production effect induced by ISCC is a persistent decrease in milk yield. The economical losses resulting from heifers with elevated ISCC, impact on the dairy farmers income. Therefore, dairy producers have a financial incentive to implement cost-effective management practices that will enable them to decrease the LSCC and maximize heifers productivity.

References:


Table 1. Distribution of LSCC classes and influence on milk production.

<table>
<thead>
<tr>
<th>ISCC Classes</th>
<th>Frequency n/(%)</th>
<th>Milk production*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≤100 x 10³ cells/ml</td>
<td>5,267 (66.23)</td>
<td>5,750 ± 250</td>
<td></td>
</tr>
<tr>
<td>101-300 x 10³ cells/ml</td>
<td>2,013 (25.31)</td>
<td>5,600 ± 150</td>
<td></td>
</tr>
<tr>
<td>301-500 x 10³ cells/ml</td>
<td>397 (4.99)</td>
<td>5,325 ± 200**</td>
<td></td>
</tr>
<tr>
<td>501-1000 x 10³ cells/ml</td>
<td>276 (3.47)</td>
<td>5,150 ± 250**</td>
<td></td>
</tr>
</tbody>
</table>

*Mean Kg 305-day lactation
**P < 0.01
**024 Mastitis incidence: The influence of farmers’ behaviour and attitudes**

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²Animal Health Service, Deventer, the Netherlands  
³Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands  
⁴Dutch Udder Health Centre UGCN, Deventer, the Netherlands  
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**Introduction:** When mastitis incidence increases, either infectious pressure increased or resistance of cows decreased. The latter can be caused by factors outside the farm (such as weather), but it usually indicates that farm management is not optimal. Numerous quantitative studies have demonstrated the effect of management practices on mastitis. However, preventive as well as treatment programs of mastitis sometimes fail for reasons that are not immediately understood by the health professionals connected to the dairy herd [4]. Several studies and papers suggest that these failures are probably explained by farmers’ dispositions and beliefs (i.e. ‘attitudes’) towards different aspects of mastitis treatment and preventive behaviour [1, 2, 4]. A recent study of Nyman et al.[3] also suggest that farmers’ attitude towards mastitis treatment and milk production influences the incidence rate of veterinary-treated mastitis more than the environmental factors such as housing conditions. However, the direct effect of farmers’ attitude on mastitis incidence is, as far as we know, not empirically investigated. Therefore, this study aims to determine and to quantify the added value of farmers’ attitudes, beyond the farmers’ behaviour, in explaining the variation of mastitis incidence, measured through the quantifiable effect of management factors.

**Materials and Methods:** As part of the Dutch Udder Health Program of the UGCN [5] 336 Dutch dairy farmers cooperated with an extensive questionnaire on self reported attitudes and behaviour regarding the prevention and treatment of mastitis. Attitude questions contained items such as: the farmers’ frame of reference, e.g. when are you satisfied with the Bulk Milk Somatic Cell Count (BMSCC) level, and farmers perceptions, e.g. is mastitis a very expensive disease? The behavioural questions contained items such as: Do you clean the udders before milking, do you fore-strip, and how often do you clean the cubicles? In addition to the survey, clinical and sub clinical mastitis data were collected. BMSCC data were collected every fortnight starting three months before the survey until 12 months after. Furthermore, the farmers were asked to register clinical mastitis cases, defined as a cow with visible abnormal milk and/or udder, for 12 months after the start of the survey. To test whether farmers’ attitudes as well as their behaviour could explain between-farm variation of mastitis incidence, linear multiple two-step regression analyses and stepwise regression analyses were conducted. In these analyses the (sub-) clinical mastitis data were used as dependent variables and the self-reported measures of attitudes and behaviour as independent variables. The mastitis data were divided in two separate dependent variables: (1) Average farm BMSCC during 3 months preceding the survey, and (2) mastitis incidence, which is a combined measure of the number of new infections of sub clinical mastitis (based on SCC) and clinical mastitis per 100 cows per farm per year. Only those attitude and behaviour measures were used in the analyses that, based on zero-order correlations, significantly related to the mastitis data.

**Results:** Results show that farmers’ self-reported behaviour and attitudes together explain respectively 44% (F (43,258) = 5.80, p<.001) and 24% (F (43,258) =2.88, p<.001) of the variation within the average farm BMSCC and the combined (sub) clinical mastitis incidence. In addition, the results of the regression analyses show that behaviour as well as attitudes are able to predict unique variance in both mastitis measures. Nonetheless, 30% of the variance
of the BMSCC measure and 10% of the variance of the combined (sub) clinical mastitis incidence measure was explained by just the attitude variables.

The stepwise regression method showed that the variation in BMSCC value was best explained by (1) the farmers' frame of reference about mastitis ($\beta=.34, p<.001$), (2) the perceived lack of control of mastitis ($\beta=.24, p<.001$) and (3) the effect of a BMSCC penalty level ($\beta=.23, p<.001$). The variation in the combined (sub) clinical mastitis incidence was best explained by (1) the frequency of contact with others ($\beta=.25, p<.001$), and (2) the effect of a BMSCC penalty level ($\beta=.24, p<.001$).

**Conclusions:** The study showed that, in addition to what farmers do, their attitude about (the prevention and treatment of) mastitis is important with respect to explaining the difference in mastitis incidence between farms. Moreover, the self-reported measures of attitude and behaviour explained more variance of BMSCC than of the combined (sub) clinical mastitis incidence.

Within the concept of attitude, especially farmers' frame of reference is important in explaining the variation in mastitis incidence. This frame of reference seems to act partly via farmers' direct perceived problem- and satisfaction levels but also indirect via penalty systems. In addition, the frequency of contact with others about mastitis seems also to predict a part of the variation in the mastitis incidence. It seems that farmers with more mastitis problems have more frequent contact with e.g. the veterinarian.

The study also showed that apparently it is easier to predict BMSCC values than mastitis incidence by self reported attitude and behaviour. An explanation could be that the "farmer factor" in addition to the "cow factor" and the "pathogen factor" is more important in BMSCC control than in mastitis incidence control, possibly because BMSCC levels can more easily be managed (e.g. by excluding high SCC milk from the tank or by treating cows with sub clinical mastitis).

Finally, it is important to note that this study was based on self reported attitudes and behaviour of farmers. It is possible that real observations of farmers’ behaviour could influence the explained variance by the used regression models. In addition, it is hard to connect causal relationships to the results of this study. Nevertheless, in spite of these limitations, this study provides an important empirical investigation into the social processes applicable to mastitis incidence and as such can be considered a good starting point for future research and also a good lead for effective communication strategies in mastitis control programs.

**References:**

P025 Bovine mononuclear leukocyte subpopulations in colostrum and peripheral blood in response to mastitis during the periparturient period

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1National Institute of Animal Health, Tsukuba, 2National Institute of Livestock and Grassland Science, Tsukuba, 3Fukuoka Agricultural Research Center, Chikushino, Japan
*Corresponding author: yokiku@affrc.go.jp

Introduction: Dairy cattle are highly susceptible to infectious diseases, like mastitis, during the period around calving because of impairment of host resistance mechanisms1. Most of the studies undertaken to understand the mechanisms of the defense of the mammary glands against pathogens particularly, have focused on the immunity mediated by mononuclear leukocyte subpopulations. Although the factors of colostrum for calves have been elucidated, the relationship between leukocyte subpopulations in colostrum and the mastitis in the periparturient period is not clearly defined.

Materials and Methods: Nine healthy Holstein cattle including 2 heifers were studied during their periparturient. The colostrum and the peripheral blood were collected from each cow at approximately 7 d prior to the predicted calving date and at 2 h, 1, 2, 3 and 7 d after calving. Evidence of mastitis in the cattle was determined using the California Mastitis Test (CMT) according to a previous report2. Using flow cytometry, we determined the expression of specific antigens on the surface of macrophages/monocytes (CD14+), T cells (CD4+, CD8+, WC1+) and B cells (CD21+) from mononuclear leukocytes in colostrum and peripheral blood of these cattle. The characteristic of leukocyte subpopulations in colostrum of mastitic and normal cattle were evaluated during the periparturient.

Results: Four of the 9 cattle scored positive on the CMT within one week after calving (mastitic group (MG)) and 5 of them scored negative (normal group (NG)). No significant differences in total leukocyte counts were found between either group during the studies. The percentage of mononuclear leukocyte subpopulations in colostrum and blood just after calving were determined. The percentage of CD14+ cells in the colostrum were significantly (P<0.01) lower in MG than in NG (Figure 1). In contrast, there were no significant differences of the other surface markers in the colostrum or the blood.

Conclusion: The CD14+ cells in colostrum play an important role of defense from the several pathogens in the mammary glands suggesting that the alteration of CD14+ cells are the marker to predict mastitis during the periparturient period. The analysis of the mononuclear leukocyte subpopulations in colostrum may be useful in clarifying the immune condition of dairy cattle suffering from peripartum mastitis.

References:
Figure 1: The percentage of CD3+, CD14+, CD25+, CD8+, CD4+, CD21+ and WC1+ cells in the colostrum just after calving in the mastitic group (gray bars) and the normal group (black bars). Values are expressed as the mean ± SEM. Asterisked mean values are significantly different (**P<0.01).
**Introduction:** The role of the membrane receptor CD14 in the heifer mammary gland was studied in mastitis induced by lipopolysaccharide (LPS), *S. aureus* and *S. uberis*. It was found that the inflammatory response of the mammary gland to LPS was associated with an expression of CD14 receptors by polymorphonuclear leukocytes (PMN) and macrophages (2, 3). In the initial phase, a lower expression of CD14 was observed (2), whereas an increasing expression of CD14 accompanied the resolution of the inflammatory process (3). Infections of the mammary gland induce an increase in the total counts of CD14+ neutrophils and CD14+ macrophages particularly. It has also been found that the expression of CD14 on PMN highly correlates with the presence of apoptotic PMN (4). CD44 is a cell surface receptor for extracellular matrix molecules. It is widely expressed in a number of tissues and cells including leukocytes (1). The CD44 plays a role in resolving inflammation (5). For example, CD44 mediates efficient phagocytosis in peritoneal macrophages (6). The object of this study was to clarify if the CD44 will be express on bovine mammary PMN and if the expression of CD14 and CD44 will be correlated in the process of resolution of inflammatory response induced by LPS and muramyldipeptide (MDP).

**Materials and Methods:** The study was performed in eight clinically healthy heifers. There was an inflammatory response of the mammary gland induced by intramammary instillation of phosphate buffered saline (PBS), which served as a control and components of the cellular wall from gram-positive bacteria - MDP and gram-negative bacteria - LPS. The mammary glands were lavaged at four time points and cell suspensions were processed for detection of surface receptors. A flow cytometer was used for the detection of CD14+ and CD44+ proportions of PMN.

**Results:** Fig. 1 shows that the highest proportion of CD14+ PMN was observed 72 hours after the induction with PBS. A statistically significant lower proportions were observed after induction with MDP (P<0.01), and after LPS (P<0.05). Decrease in the proportion of CD14+ PMN followed. The similar pattern was observed in CD44 expression (Fig. 2). Compared with MDP and LPS there was a statistically highly significant (P<0.01) lower proportion of CD44+ PMN observed within 168 hours after induction with PBS. As indicated in Table 1, a statistically significant correlation was demonstrated between proportions of CD14+ and CD44+ PMN during all experimental period after the induction with MDP. Induction with LPS caused statistically significant correlation only during initial phase of inflammatory response.

**Conclusion:** Bovine mammary gland PMN expressed both CD14 and CD44 surface receptors in course of inflammatory response induced by LPS and MDP. A pattern of CD14 and CD44 expression dynamic was similar for MDP, LPS and PBS. The highest proportions of CD14+ and CD44+ PMN were observed on start of resolution process. Expressions of CD14 and particularly CD44 on PMN are significantly lower after inflammatory induction by MDP and LPS than after PBS. This indicates that expression of CD14 and CD44 on PMN is dependent on the factors inducing inflammatory response as well as on the mechanisms of resolution.

**References:****


This study was supported by Czech Science Foundation (GAČR No. 524/03/1531) and by the Ministry of Agriculture of the Czech Republic (MZE 0002716201).

**Fig. 1:** Proportion of CD14+ PMN

**Fig. 2:** Proportion of CD44+ PMN

**Table 1.** Correlation between proportions of CD14+ and CD44+ polymorphonuclear leukocytes

<table>
<thead>
<tr>
<th>Inflammatory inducers</th>
<th>Time points</th>
<th>Correlation</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[ hours]</td>
<td>[ r²]</td>
<td></td>
</tr>
<tr>
<td>MDP</td>
<td>24</td>
<td>0.582</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.881</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.929</td>
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</tr>
<tr>
<td></td>
<td>168</td>
<td>0.810</td>
<td>**</td>
</tr>
<tr>
<td>LPS</td>
<td>24</td>
<td>0.952</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.690</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.396</td>
<td>N.S.</td>
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<tr>
<td></td>
<td>168</td>
<td>0.429</td>
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</tr>
</tbody>
</table>

Significance * P<0.05; ** P<0.01; N.S. none significance

MDP muramyldipeptide; LPS lipopolysaccharide
P 027 Variation in gene expression of the TLR4 pathway in unstimulated circulating bovine neutrophils during early and mid-to-late lactation

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2 Drug Quality & Registration (DruQuaR) Group, Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Gent, Belgium
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Introduction: It is well known that mammary E. coli infections in lactating cows cure spontaneously during mid and late lactation. Clinical signs may vary from mild to moderate. During early lactation, spontaneous cure occurs less frequently, the inflammatory reaction is much more severe and fatal sepsis is not exceptional6. Several authors have suggested that the variation in clinical signs is related to an impairment of the efferent arm of innate defense. More specifically, polymorphonuclear neutrophil leukocyte (PMN) function may be altered at parturition and during early lactation. In particular, slow PMN diapedesis and reduced chemotaxis2,3, and decreased oxidative burst5-8 may contribute to the severity of coliform mastitis. Recent studies have suggested that early sensing of the intramammary infection with E.coli, may also be critical in the pathogenesis of E.coli mastitis. Toll-like receptor 4 (TLR4), is an important constituent of the afferent arm of innate immunity. This receptor functions as an LPS-sensor. It recognises lipopolysaccharides (LPS). LPS is released during growth and lysis of E.coli. Via multiple signalling cascades, LPS induces expression of several inflammatory cytokines: e.g. IL1, IL6 and IL8. Sensing the LPS too late may result in an uncontrolled inflammatory reaction. It has been reported that several PMN functions may vary depending on the stage of lactation. In the present study, the expression of genes related to the TLR4 pathway was studied in isolated bovine PMN’s. Unstimulated PMN’s were collected from the jugular vein during early and mid-to-late lactation. Following genes, using quantitative PCR (qPCR), were analysed: TLR4, MyD88, IKKα, IKKε, the NF-κB p65 subunit, TRAF6, MD2, ATF3, JNK1, Syk, TAK1, PI3Kα, TRIAD3a, TRIF and CD14.

Materials and methods: PMN’s were isolated from blood collected by venipucture from early (5-32 days) and mid to late (189-218 days) lactating cows, using the hypotonic lysis procedure. The cows were free from udder infections and had a SCC below 200.000. Following the RNA extraction with TRIR, chloroform and isopropanol, cDNA was synthesised with the iScript Reverse Transcriptase kit (Biorad). After a control PCR to check if the cDNA synthesis was successful, the expression of the mentioned genes was quantified with qPCR. qPCR was performed using Sybr Green and the 3 reference genes selected by De Ketelaere4.

Results and discussion: Following sequential particularities in gene expression were observed between animals in early and mid-to-late lactation. 1) Although no differences could be detected in TLR4 gene expression, early lactating cows showed a significant higher expression of downstream bovine PMN’s. Unstimulated PMN’s were collected from the jugular vein during early and mid-to-late lactation. Following genes, using quantitative PCR (qPCR), were analysed: TLR4, MyD88, IKKα, IKKε, the NF-κB p65 subunit, TRAF6, MD2, ATF3, JNK1, Syk, TAK1, PI3Kα, TRIAD3a, TRIF and CD14.

Materials and methods: PMN’s were isolated from blood collected by venipucture from early (5-32 days) and mid to late (189-218 days) lactating cows, using the hypotonic lysis procedure. The cows were free from udder infections and had a SCC below 200.000. Following the RNA extraction with TRIR, chloroform and isopropanol, cDNA was synthesised with the iScript Reverse Transcriptase kit (Biorad). After a control PCR to check if the cDNA synthesis was successful, the expression of the mentioned genes was quantified with qPCR. qPCR was performed using Sybr Green and the 3 reference genes selected by De Ketelaere4.

Results and discussion: Following sequential particularities in gene expression were observed between animals in early and mid-to-late lactation. 1) Although no differences could be detected in TLR4 gene expression, early lactating cows showed a significant higher expression of downstream MyD88. 3) Many genes located downstream TLR4 and MyD88 (except CD14 and MD2), showed similar gene expressions. 4) Gene expression of Trif was significantly (p = 0.0311) higher during early lactation. Since this protein plays a central role in both the MyD88-dependent and independent TLR4 pathway1, increased expression of this gene during early lactation would imply a increased TLR4 signaling resulting in a higher expression of inflammatory cytokines. 5) The difference in expression for IKKε and Syk was...
not significant although there was a tendency for a lower expression of both genes during early lactation. Syk is necessary for the migration of the NF-κB p65 subunit to the nucleus and is involved in the regulation of the β2 expression (CD11/CD18) and NO production. This protein is also needed for the activation of the transcription factor of AP1 which is involved in protection against apoptosis. IKKε has been shown to phosphorylate IRF3, which enables IRF3 to migrate to the nucleus where it induces the expression of inflammatory genes like interferon-β and the chemoattractant RANTES. Theoretically, a lower expression of Syk and IKKε in early lactation can cause a reduction in PMN recruiting to the site of inflammation, a reduced activation and a higher apoptosis rate of these cells and a lowered NO, IL1 and IL6 production in comparison to mid-to-late lactation. This could partly explain why the upregulated Trif expression doesn’t result in an increased TLR4 signalling and thus a stronger immune response in early lactation.

Conclusion: Differences have been shown in some gene expression downstream the TLR-4 gene, in unstimulated PMN isolated during early and late lactation. The expression of Trif and MyD88 was significantly upregulated in early lactation. IKKε and Syk gene expression of showed a tendency to be reduced during early lactation. To what extent these differences are involved in the altered PMN function observed at parturition and during early lactation, remains to be resolved.

References
P 028 The prevalence of Heifer Mastitis due to Leptospirosis

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Introduction: Leptospirosis is an infectious zoonotic disease which occurs by members of the genus Leptospira. The pathogenic Leptospira are classified into one species of Leptospira interrogans (L. I.) containing over 212 serovars arranged into 23 serogroups. The Leptospira antibodies have been detected in domestic animals and human in Iran. Leptospirosis in cattle may be acute, subacute or chronic. Mastitis is one of the signs of leptospirosis. The objectives of this study were: 1) to evaluate the occurrence of heifer mastitis in an outbreak of leptospirosis in a dairy cattle herd complex. 2) If there are differences between primiparous and multiparous cows mastitis due to leptospirosis.

Material and Methods: Leptospirosis was occurred in a large industrial dairy cattle herd complex in Iran during 2 years. It was included about 760 heifers and multiparous Holstein dairy cows. The disease initiated with clinical mastitis. The other signs of leptospirosis were septicemia, high fever, anorexia, hemolytic anemia, jaundice, abortion, hemoglobinuria and pallor of the mucosa. The milk production dropped markedly. The sudden drop in milk production affected more than 50% of cows and caused a precipitation fall in the herd milk. The heifers were in 5-90 days of post partum. The udder was flabby and there was no heat or pain and all four quarters were equally affected. The appearance of mastitis was blood-stained or yellow, thick milk in all four quarters (systemic mastitis). The decline lasted for up to 6 weeks, but individually milk production returned to normal within 10-14 days. The Microscopic Agglutination Test (MAT) used for the Leptospira diagnosis. Sera were screened against 22 alive antigens. A MAT titer of ≥1:100 were considered positive. During 2 years leptospirosis outbreak, the control and treatment strategies limited the occurrence. In brief they were included: 1) Identification of heifers and other cows with clinical finding and subsequent treatment with dihydrostreptomycin (12.5 mg/kg, IM) twice daily for 3 days. 2) Antibiotic therapy of the entire herd with no clinical signs at the one time with dihydrostreptomycin (25 mg/kg, IM). 3) Vaccination of all cows with Leptospira bacterin contained mentioned below serovars and repeated after 6 months then followed by annual revaccination. 4) Implementation of tight hygiene management such as reduction of rat and wildlife population, and keeping the corrals in dry condition. The somatic cells were counted by Fossomatic. Data were analyzed by using Chi-square statistic method.

Results: The MAT showed that the heifers were infected by L. I. Pomona, Hardjo, Grippothypyosa, Icterohaemorrhagiae and Canicola serovars. In total, 76 (18.71%) fresh calved heifers out of 406 infected dairy cows showed the symptoms of mastitis during 2 years (Table 1). The prevalence of leptospiral clinical mastitis was significantly lower than multiparous dairy cows (P<0.05). The titer of MAT was between 1:100 to 1:3200. Nine heifers (2.21%) showed the mastitis signs for 2 times. The somatic cell count ranged about 10,000,000.

Discussion: Mastitis caused by leptospirosis has often been described in cattle, resulting from L.I. Hardjo and Pomona1. In endemically infected dairy herds, there may be no relationship between seropositive and seronegative cows in different lactation. However, this study revealed that the prevalence of mastitis in heifers is significantly lower than multiparous cows (P<0.05) (Table 1). The seroprevalence of infected heifers were more than the heifers that showed the leptospiral mastitis signs. A high somatic cell count in grossly abnormal milk suggests mastitis in the heifers. The mammary gland changes are due to a general vascular lesion rather than local injury to mammary tissue. Since the organism can be proliferated in...
mammary gland, transmission via infected milk\(^1\) and the sudden drop of milk caused economical losses. Therefore, the application of control and treatment programs can limit this zoonotic disease. It is concluded that 1) leptospiral mastitis in primiparous cows are significantly lower than multiparous cows. 2) Mastitis is one of the most important signs of leptospirosis in fresh calved heifers and multiparous dairy cows. 3) It can be limited by preventive sanitation programmes. More studies should be done in the field of heifer mastitis due to leptospirosis.

**References**


**Table 1: Prevalence of heifer and multiparous Holstein dairy cow mastitis due to leptospirosis**

<table>
<thead>
<tr>
<th>The year of duration of outbreak</th>
<th>1(^{st}) lactation No. (Heifers) (%)</th>
<th>&gt;1 lactation No. (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47 (11.69%)</td>
<td>310 (76.35%)*</td>
<td>357 (87.93%)</td>
</tr>
<tr>
<td>2</td>
<td>29 (7.14%)</td>
<td>20 (4.92%)</td>
<td>49 (12.06%)</td>
</tr>
<tr>
<td>Total</td>
<td>76 (18.71%)</td>
<td>330 (81.28%)</td>
<td>406</td>
</tr>
</tbody>
</table>

*Significant differences (P<0.05)
P 029 A Study of the Incidence of Bacterial Species Associated With Intramammary infections in Dairy Heifers in Mashhad, Iran

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Introduction: It is now understood that dairy heifers are at risk for developing mastitis early in life, even before attaining breeding age. Studies have documented mastitis in heifers as young as 9 month of age, and investigations have shown that infection rates can be as high as 97%. The staphylococci are the predominant mastitis pathogens causing heifer mastitis, and Staphylococcus aureus can represent a substantial percentage of these infections. Other studies showed a high proportion of CNS in quarter samples from heifers with prepartum clinical mastitis. The purpose of the present study was to identify microorganisms that cause mastitis in heifers in some dairy industry farms of Mashhad, Iran.

Materials and Methods: In this study 245 quarter secretion samples from heifers of prepartum or within 20 d postpartum were examined bacteriologically. Quarter secretions were examined according to the methods and procedures employed by the Mastitis Laboratories of the National Veterinary Institute, which are in agreement with the recommendations of the International Dairy Federation. For samples taken postpartum, the cell count was estimated using the California Mastitis Test. 0.1 ml of Secretions was plated onto trypticase soy blood agar plates (TBA) containing 0.1% esculin (Sigma) and 5% calf blood. Quarter samples were plated on TBA. Plates were incubated at 37°C and colony characteristics and numbers of colony forming units were recorded at 48 h. Gram stain and culture characteristics on TBA (i.e., colony morphology, pigmentation, aroma, hemolysis, and catalase production) were used for presumptive identification for all isolates. Species identification of staphylococci was performed using the API Staph-Trac system. Streptococci were identified as by Watts (1988).

Results: Result of this study showed that 40% of quarters affected with one or more microorganisms. The organisms that were most frequently isolated from samples from these quarters were Staphylococcus aureus (11.72%), Streptococcus agalactiae (4.14%), Streptococcus dysgalactiae (8.27%), Staph. aureus together with Strep. dysgalactiae (5.52%), coagulase-negative staphylococci (66.21%), Streptococcus bovis (2.76%), and Escherichia coli (1.38%). Of the coagulase-negative staphylococci, Staphylococcus saprophyticus (8.33%), Staphylococcus epidermidis (18.75%), Staphylococcus haemolyticus (27.08%), Staphylococcus hyicus (2.08%) and Staphylococcus chromogenes (43.75%), were the most prevalent species. No significant differences were observed in the distribution of the various organisms among prepartum and postpartum cases (P> 0.05). Results of the present study showed that coagulase-negative staphylococci, are the most frequent isolates.

Conclusion: In general, CNS are less pathogenic than are most other organisms associated with bovine mastitis. Of the CNS associated with clinical mastitis in heifers, there are indications that Staph. hyicus is more pathogenic than other species, although other reports on bovine mastitis indicate that there are no differences in pathogenicity among different CNS species. Therefore, to minimize the economic losses to dairy fanners, it is important to know why some heifers have clinical mastitis at calving and how mastitis affects their productivity.

References:
P030 Pro- and anti-inflammatory effects of in vitro Escherichia coli phagocytosis by bovine neutrophils

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Introduction: Escherichia coli (E. coli) is an environmental pathogen causing acute bovine clinical intramammary infections. Bovine neutrophils are known to play a key role in the early host defense mechanisms towards coliform mastitis. After recruitment to the site of infection, these cells recognize the invading pathogen which is then internalized and destroyed. In human neutrophils, phagocytosis triggers pro- and anti-inflammatory intracellular signaling cascades, thereby regulating the innate immune response. Promoting both the clearance of microbial infections as well as the resolution of inflammation by inducing neutrophil apoptosis are major consequences of pathogen invasion and recognition. For bovine neutrophils, molecular events following phagocytosis are rarely described. The objective of this study was therefore to elucidate some early and late effects of E. coli phagocytosis by bovine neutrophils.

Material and Methods: Peripheral blood samples were collected from healthy heifers. Bovine neutrophils were isolated following red blood cell lysis by ammonium chloride and density gradient centrifugation with Percoll. Highly purified cells were incubated at 37°C with an E. coli strain isolated from a bovine clinical mastitis case. Various E. coli/PMN ratios were tested. At different time points, cytospins were made to confirm phagocytosis and apoptosis. In addition, apoptotic neutrophils were quantified by the flow cytometric detection of exposed phosphatidylserine. Medium was collected for cytokine analysis of secreted interleukin-1 (IL-1) and interleukin-6 (IL-6) via bio-assays. Protein lysates were prepared for use in the TransAM™ nuclear factor-κB (NF-κB) p65 ELISA kit.

Results: At early time points, the transcription factor NF-κB p65 was activated in neutrophils following incubation with E. coli bacteria (Fig. 1). Secreted IL-1 and IL-6 cytokine levels were elevated upon phagocytosis, whereas no cytokines were released by control cells. At later time points, the percentage of apoptotic neutrophils was increased in the presence of E. coli as compared to control (Fig. 2).

Conclusions: We conclude that in vitro E. coli phagocytosis by bovine neutrophils initially leads to pro-inflammatory events such as the activation of the transcription factor NF-κB, a key component within the context of immune responses. Subsequently, secretion occurs of IL-1 and IL-6, pro-inflammatory proteins of which the transcription is regulated by NF-κB. On the contrary, at later time points, neutrophils are promoted to end their useful lifespan. Phagocytosis of E. coli by bovine neutrophils thus activates competing pathways which initially favor combat of the pathogen, but ultimately trigger immune cell death.

References:
Figure 1: Representative example of NF-κB p65 activity in bovine neutrophils following incubation with E. coli after isolation and after 10, 20 and 30 minutes (min) of incubation (ratio 5/1).

Figure 2: Effect of E. coli phagocytosis on cell surface exposure of phosphatidylserine in bovine neutrophils after isolation and after 3, 6 and 24 hours (h) of incubation (ratio 50/1). Data are means ± SEM of 6 heifers.
P031 Associations between management practices in the period around parturition and somatic cell counts of primiparous cows in early lactation

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Introduction: Several studies have found aspects of housing, milking and management to be associated with udder health of dairy cows1,2,3,4. However, all these studies have included both primiparous and multiparous cows. Heifers, and to some extent primiparous cows, are often housed in different housing systems, are managed differently than milking cows, and have never experienced milking. The period around parturition is stressful and management factors might have more pronounced effects on primiparous than on multiparous cows. Thus, it is plausible that there are different risk factors in housing, milking and management in the period around parturition for primiparous cows compared to multiparous cows. The objective of the current study was therefore to investigate associations between management, milking, and housing around parturition, and somatic cell counts (SCC) of primiparous cows in early lactation.

Materials and Methods: An observational study of 72 large (≥80 cows), high producing, low SCC dairy herds housed in free stalls was performed in Sweden from October 2005 to March 2006. Information on management and housing system one month before, at and at one month after parturition was collected, as well as milking routines during and after the colostrum period. Data on herd size, average milk production, udder-disease scores and breed composition of the herd was collected from the Swedish official milk recording scheme (SOMRS). The SOMRS also provided individual data, for the 1,189 primiparous cows calving during the study period, on breed, age at calving, and SCC and milk yield at first test-milking. The SCC were analysed on herd level as number of cows with a SCC ≥200,000 cells/ml milk at first test-milking, and at individual cow level as the lnSCC at first test-milking. Poisson regression analysis was used to study risk factors at the herd level, while hierarchical linear regression analysis, with herd as random factor, was used to study risk factors at the individual cow level. Associations between the dependent variable and each of the potential risk factors were first screened in univariable Poisson or linear regression analysis. Variables with a P-value ≤0.20, provided that there was no collinearity (r<0.70) between variables, were then considered for further analysis. Only variables with P-values ≤0.05 remained in the final models.

Results: Herd size, geometric mean bulk milk SCC, and milk production for the 72 herds during the year of participation (September 1, 2005 to August 31, 2006) were 138 cows/year, 196,000 cells/ml, and 10,130kg milk/cow/year, respectively. The geometric mean SCC at first-test milking (occurring on average 20 days postpartum) was 64,300 cells/ml (50% central range: 29,000 - 118,000 cells/ml), and 175 primiparous cows (14.7%; range 0-14 primiparous cows/herd) from 58 herds had a SCC ≥200,000 cells/ml at first-test milking. A total of 152 herd level variables and 11 individual cow level variables were screened in the initial univariable analyses for associations with SCC. Descriptive data showed that the primiparous cows were housed in free stalls in 74% of the herds, and in tie stalls in 26% of the herds at one month before calving. Most commonly primiparous cows
calved alone or in group in a calving box (in 68% and in 17% of the herds, respectively). They were moved to the calving area in ≤1 day before calving in 61% of the herds and from the calving area in ≤1 day after calving in 54% of the herds. In 43% of the herds the primiparous cows went to the parlor before calving to get used to the milking routines, while in 40% of the herds no adaptation to milking was applied. Primiparous cows were milked in the calving area during the colostrum period in 24% of the herds. Post-milking teat disinfection was used in 78% of the herds in the colostrum period and in 87.5% of the herds after the colostrum period. Only 3 variables remained with P-values ≤0.05 in the final models (Table 1). Management factors significantly associated with an increased lnSCC at first-test milking was to rinse, clean or disinfect the milking units before a primiparous cow was milked, as well as moving primiparous cows from the calving area to the milk cow area in ≥2 days after calving. Rinsing, cleaning or disinfecting the milking units before a primiparous cow was milked was also associated with an increasing number of primiparous cows with a SCC ≥200,000 cells/ml at first-test milking. A low proportion of cows in the herd with high SCC was associated with decreasing number of primiparous cows with a SCC ≥200,000 cells/ml at first-test milking. The intra class correlation of cows between herds, calculated from the lnSCC model, was 0.19 (0.21/1.08), consequently, most unexplained variation were between cows in the same herd.

Conclusion: In the present study only a few risk factors was found associated with SCC of primiparous cows probably due to too similar herds and that nearly all risk factors was measured on herd or group level. Most of the unexplained variation in lnSCC was at the individual cow level, thus, to be able to reduce the lnSCC of primiparous cows further studies of individual animal risk factors are needed.

References:
### Table 1: Final models of the multivariable Poisson-regression analysis of risk factors significantly (P≤0.05) associated with number of primiparous cows with a somatic cell count (SCC) ≥200,000 cells/ml at first test-milking, and of the hierarchical multivariable linear-regression analysis of risk factors significantly (P≤0.05) associated with lnSCC of primiparous cows at first test-milking in 72 Swedish dairy herds.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>No. of animals</th>
<th>LSM$^1$</th>
<th>No. of herds</th>
<th>IRR$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of cows with udder-disease score (UDS)</td>
<td>1: ≥22%</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>Ref.</td>
</tr>
<tr>
<td></td>
<td>2: 13-21.9%</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td>0.81</td>
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<tr>
<td></td>
<td>3: &lt;13%</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>0.62</td>
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<td>Milking units are rinsed, cleaned or disinfected before a first-parity cow is milked</td>
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<td>57.2</td>
<td>49</td>
<td>Ref.</td>
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<tr>
<td></td>
<td>2: Yes</td>
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<td>76.7</td>
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<td>1.67</td>
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<tr>
<td>Moved from the calving-area</td>
<td>1: ≤1 day after calving</td>
<td>561</td>
<td>56.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2: ≥2 days after calving</td>
<td>628</td>
<td>70.8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^1$LSM=least square means back transformed to original scale and expressed as SCCx1000/ml

$^2$IRR = incidence rate ratio

$^3$Animals with UDS 6-9 have a probability of 69.99% that one or more udder quarters are infected. The score corresponds approximately to having a SCC of >300,000 cells/ml on three consecutive test-milking.
P032 Molecular analysis of ‘no growth’ milk cultures from mastitic cows

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Introduction

Bovine mastitis is a multifactorial disease and one of the most difficult pathologies to control. Identification of the bacterial pathogens in milk from cows with mastitis is regarded as the definitive diagnosis of mastitis infection. However, in many cases (up to 30%) no aerobic bacteria can be cultured from the milk3. Reasons given for these ‘no growth’ cultures include low numbers of organisms in the milk, inadequate growth conditions for the causative pathogen, intermittent shedding patterns, mishandling of samples during storage and the presence of inhibitors such as antibiotics4. Nucleic acid-based methods are highly sensitive and specific, being able to detect pathogens at lower concentrations than conventional microbiological methods. They can also discriminate between closely related organisms such as Streptococcal species. The application of molecular methods to unculturable milk samples from mastitis animals could facilitate the identification of a causative agent and indicate the source of infection. In addition, it may also influence the course of treatment that is taken.

Materials and Methods

Milk Samples: A total of 46 milk samples from cows presenting with clinical mastitis, or associated with elevated somatic cell counts (SCC) were analysed in this study. These samples were collected and analysed using the standard procedures recommended by the International Dairy Federation (IDF). Samples included eighteen milk samples from which specific pathogens were cultured. Preliminary identification of these pathogens was by colony morphology, hemolysis on blood agar, biochemical tests and Gram staining. Twenty eight milk samples were from clinical mastitis cases that were recorded as ‘no growth’, as no pathogen was cultured from the samples. All of the milk samples were stored at -20ºC prior to analysis by molecular methods.

Bacterial strains: The control strains used in the study included three typed strains, Staphylococcus aureus (ATCC12600), Streptococcus uberis (LMG14898), Streptococcus parauberis (LMG12174), two clinical isolates from confirmed bovine mastitis cases Streptococcus dysgalactiae (DPC5345), Streptococcus agalactiae (DPC5338) and an E.coli strain (DPC6473) DNA from Mycoplasma bovis species was kindly donated by Edy Vilei of Bern University, Switzerland.

DNA extraction procedures
DNA was directly extracted from 1mL of milk using the procedure recommended by Cremonesi et al1. Bacterial DNA was extracted from 5mL pure cultures, grown overnight at 37ºC in TSB (Oxoid Ltd., UK), using the QIAmp DNA mini kit (QIAGEN Ltd., UK).

Polymerase Chain reaction (PCR) analysis
Milk DNA samples were initially analysed using the primer pair Uni1870 (5'-TGGAAGGT TAAGGGAGTG) and Uni2308 (5'-GCTCCGTTACCTTTAGGA), as described by Riffon et al6. These primers were designed from the DNA regions coding for the 23S rRNA sequence and amplify DNA from the six most prevalent bacteria known to cause bovine mastitis, including Escherichia coli, Staphylococcus aureus, Streptococcus uberis, Streptococcus parauberis, Streptococcus agalactiae, and Streptococcus dysgalactiae. Species-specific primers described by Riffon et al.,6 and Forsman et al.,2 were also used to identify the species of each organism present in the milk. In addition, primers described by Pinnow et al.,5 were used to investigate for the presence of Mycoplasma bovis DNA. PCR
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assays were optimised using known pathogen DNA and all products were visualised by agarose gel electrophoresis and ethidium bromide staining.

Results
Pathogen DNA was effectively extracted from all milk samples, as evidenced by the amplification of PCR products from each sample. Using the primer pair Uni-1870 and Uni-2308 a 438bp product was amplified from 42 of the 46 samples. Species-specific primers were applied to all of the samples to amplify pathogen-specific DNA. From the 18 culturable milk samples pathogen DNA was amplified that correctly corresponded to the microbiological data. Analysis of the 28 'no growth' cultures revealed that some samples contained a single pathogen while others contained mixed pathogen DNA. Amplification with the Staphylococcus genus primers confirmed Staphylococcal DNA in 17 samples and Staphylococcus aureus DNA was confirmed in 3 of these samples. Coliform DNA was detected in 11 of the 28 milk samples. The predominant DNA present in the 28 milk samples was from Streptococcal species, with Streptococcus uberis found in the majority (23/28) of samples. Six of these samples also contained DNA from Streptococcus dysgalactiae. A single sample contained DNA from a Streptococcus agalactiae species mixed with Streptococcus uberis DNA. PCR analysis using Mycoplasma bovis-specific primers did not amplify DNA from this species in any of the milk samples.

Conclusions
Mastitis pathogen DNA was amplified from 28 milk cultures that were previously recorded as 'no growth' cultures. The predominant organism detected was Streptococcus uberis, as 23 of the 28 milk samples contained DNA from this pathogen. DNA from Staphylococcus aureus was detected in three of the clinical mastitis milk samples. DNA from the Mycoplasma bovis species was not detected in any of the milk samples tested. The PCR-based methods were fast (result in <1 day), sensitive and reproducible. This preliminary study indicates that molecular methods such as PCR can be used to identify pathogen DNA in clinical mastitis cases where there is no pathogen detected using conventional microbiological methods. A study is now underway to confirm the reproducibility of this molecular tool to consistently identify mastitis pathogen DNA in 'no growth' cultures and compare its performance to conventional microbiological methods.

References
**P033 Incidence of intramammary infection at parturition for first calf heifers and multiparous cows**

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**Introduction**
Intramammary infection by *Staphylococcus aureus* continues to be a major concern among dairymen. Despite adoption of management practices such as pre- and postmilking teat dipping, dry cow therapy and culling of infected animals, dairymen continue to have problems eliminating intramammary infections by *S. aureus* from their herds. While the general perception among dairymen is that mastitis in fresh heifers is very low, it has been reported that intramammary infection of fresh heifers provides a source for the introduction of *S. aureus* into herds (1). We have observed a recent increase in the incidence of clinical mastitis caused by *S. aureus* in the Beltsville Agricultural Research Center (BARC) research dairy herd. The herd is well-managed and cows are teat dipped pre- and postmilking and treated with antibiotics at dry-off for the control of contagious pathogens. To determine if fresh heifers were serving as a source of *S. aureus*, the present study was conducted to determine the incidence of intramammary infection at the time of parturition for first calf heifers and multiparous cows.

**Materials and Methods**
Aseptic quarter foremilk samples (10 ml) were obtained within 3 days of calving from 116 heifers (463 quarters) and 101 multiparous cows (404 quarters) from October 2003 to the present. For the herd, calving occurred year round. Samples were collected aseptically after disinfection of teat ends before preparing the udder for milking. Diagnostic bacteriology was conducted by spreading 20 µl of milk over the surface of an esculin blood (calf) agar plate. Resulting bacterial colonies were observed after 24 and 48 hours of incubation. Colonies were classified according to procedures established by the National Mastitis Council (2).

**Results**
Intramammary infections were observed in 183 quarters (40%) of 87 heifers, and in 117 quarters (29%) of 59 multiparous cows. Of the 183 infected quarters for heifers, 117 (64%) were infected with coagulase negative staphylococci, 22 (12%) with Gram-negative bacteria, 31 (17%) with *S. aureus*, 4 (2%) with *Streptococcus* spp, and 9 (5%) with *Corynebacterium bovis*. For multiparous cows, 77 (66%) were infected with coagulase negative staphylococci, 16 (14%) with Gram-negative bacteria, 8 (7%) with *S. aureus*, 6 (5%) with *Streptococcus* spp, and 5 (4%) with *C. bovis*. For all cows, the data indicate a prevalence of 7% and 2%, respectively, in the quarters of fresh heifers and multiparous cows infected with *S. aureus*.

**Conclusions**
Coagulase negative staphylococci accounted for the majority of bacteria isolated from quarters of both fresh heifers and multiparous cows. A greater prevalence of intramammary infections by *S. aureus* was observed in fresh heifers than in multiparous cows. Fresh heifers are a source for the introduction of *S. aureus* intramammary infections into the BARC dairy herd.

**References**
A critical evaluation of the control of clinical and subclinical mastitis in heifers in pasture-based Australian dairy herds

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Introduction

Over the last ten years or so, the Australian dairy industry has supported a national mastitis control program that has aimed to educate farmers of the principles of mastitis control and to set achievable performance targets to assist them in improving the quality of milk supplied by their herd. This program, known as “Countdown Downunder”, provides a variety of advice to farmers on how to monitor, treat and prevent mastitis in heifers, based on a well documented, critical review of the available literature (Brightling et al 2000).

Components of this program specifically relating to heifer mastitis include advice on methods of purchasing heifers as well as ways to monitor clinical and subclinical mastitis rates in heifers. In addition, advice is provided dealing with aspects of calving management, early lactation management and treatment of mastitis in heifers that can help to reduce the incidence of mastitis in this group of animals within the herd.

Substantial progress has been made by the Australian dairy industry in the implementation of improved mastitis control practices in recent years. This has been despite of, or perhaps partially as a result of, extremely harsh environmental and economic conditions in the country that have driven many dairy farmers off their farms. Economic factors are powerful motivators of changed farmer behaviour in Australia as they are elsewhere in the world (Ruegg and Rodrigues 2007, Shukken et al 2007).

Despite the establishment of the Countdown Downunder program, and the continued emphasis by advisers of the importance of milk quality to farmers, there is a broad perception that mastitis in heifers continues to be an area of weakness in the control of this disease in Australia. The factors which increase the risk of mastitis in heifers in a predominantly pasture-based farming system are not well defined (Parker et al 2007). This has lead to interest in a variety of methods by which new infections can be prevented in heifers, or by which existing infections can be quickly and efficiently cured. (Beggs and Wraith 2006, Parker et al 2007, Borm et al 2006)

Materials and Methods

Owners of commercial seasonal calving dairy herds in a region of south west Victoria (in south east Australia) were asked to provide data relating to the occurrence of clinical and subclinical mastitis in heifers and adult milking cows in their herds during the 2006-07 lactation season. Selection of herds for inclusion in the study was not random but was based on an active relationship with the local veterinary practice. Herds for which there was believed to be good quality mastitis records were specifically targeted for inclusions in the study. Herd owners were initially approached by letter, with follow-up contact by telephone. A description of the herd quality assurance records and stock treatment records of each farm was requested. Quantitative data for each herd was collected for the heifer replacement rate, rates of clinical and subclinical mastitis in heifers, and the comparable rates of mastitis in adult milkers. Herd owners were also asked to provide information on the rate of culling in their herd of heifers due to mastitis.

Farmers were further asked to describe their approach to culling decisions with heifers affected by mastitis and how their herd performance compared with targets set by the Australian national mastitis and cell count program (“Countdown Downunder”). Each herd
owner provided their individual assessment of the economic impact to their business caused by heifers not completing their first lactation due to clinical mastitis.

Results

Heifer replacement rates varied substantially between herds, ranging from 14 - 21%. There was a large variation between herds in the percentage of heifer clinical cases detected and treated within 7 days of an individual heifer’s calving event. The incidence of clinical mastitis in heifers was significantly lower than that in adult milking cows from the same herd.

In the majority of herds mastitis was only one of the reasons for culling heifers. In only one of the herds studied was mastitis the sole reason for heifer culling decisions within the group. There was little consistency between farms in how they monitored the occurrence of mastitis within their herd. Similarly, farmers used many different strategies to decide whether to cull a heifer on the basis of mastitis.

Conclusions

Under current Australian conditions there is no regulatory requirement for dairy farms to maintain clinical mastitis records. There is, however, a requirement by all three major regional milk processors that mastitis information be recorded in an on-farm quality assurance database primarily for the purposes of prevention of antibiotic contamination of bulk vat milk. The validity and accuracy of this information varies enormously between farms. A comprehensive assessment of the human and farm factors leading to incomplete record keeping is difficult. Milk recording and individual cell count analysis is only performed on 52% of Australian herds so this information is non-existent in many herds.

Under Australian conditions the vast majority of heifers have not yet returned their rearing costs to the production system at the end of their first lactation. Hence the loss of an animal due to a clinical mastitis event, which in itself has a calculable cost, represents a net loss to that business. Despite this, many farmers do not recognise the importance of mastitis in this group of animals and do not have good systems in place to detect the emergence of a mastitis problem in this group specifically.

It is difficult to maintain the focus of dairy producers on the importance of milk quality in the face of the multitude of other threats that challenge the farm manager on a daily basis. It is important that farmers are not only given well defined targets to aim for, but are also encouraged to implement systems by which they can not only accurately monitor the performance of their herd as a whole, but can monitor the performance of critical groups of animals within their herd. Their remains considerable scope for the development of improved strategies to assist the decision making process when deciding whether to cull heifers on the grounds of mastitis.

References

P035 Energy balance of primiparous and multiparous Holstein cows following short dry periods

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Introduction: Nowadays, a 51-60 days (d) dry period (DP) for high yielding cows is an acceptable management constant worldwide. Recent literature shows that a 30-40 d DP is sufficient for maximizing the number of differentiated secretory cells. In itself, shortening the DP, minimizes the dietary changes frequencies. Consequently, by shortening the DP, the nutritional stress is also expected to decrease. Further, a short DP may also improve the survival of the desirable population of rumen flora. Regardless of the productive ability of cows subjected to a short DP, the energy balance remains an important factor. The objective of current study was to assess the energy status of dairy cows subjected to a short DP. Cows were subjected to three different DP treatments: 1) a traditional DP (56d), 2) an intermediate DP (42d) and 3) a short DP (35 d).

Materials and Methods: One hundred and twenty two Holstein cows were included in this study. Cows were subjected randomly in a 3×2 factorial arrangement to a completely randomized design as following: 1) traditional DP (56d) for primiparous (TP) and multiparous (TM) cows, 2) intermediate DP (42d) for primiparous (IP) and multiparous (IM) cows and 3) short DP (35 d) for primiparous (SP) and multiparous (SM) cows. Actual DP lengths for respective treatments were 56 ± 5.1d, 42 ± 2.1d & 35 ± 2.7d. Body condition score (BCS) was evaluated at dry-off, calving and 15 weeks of lactation during the experiment by one evaluator. Blood samples (10 ml) were collected from the coccygeal vein (tail vein) before expected calving and immediately after parturition. NEFA (NEFA-C, Wako Chemicals USA, Inc., Richmond, VA) concentrations were measured using colorimetric enzymatic reactions with an automated wet chemistry analyzer (Hitachi 917, Roche Diagnostics, Indianapolis, IN). Total plasma TG concentrations were measured by using spectrophotometric analysis and a commercially available enzymatic kit (Sigma Chemical, St. Louis, M). Serum glucose samples were analyzed by a commercial kit (Sigma kit Trinder: Sigma Chemical Co.). IGF-1 was measured by double antibody immunoradiometric assays (IRMA) using reagents from Immunotech (Marseille, France). Serum insulin concentration was measured by radioimmunoassay. During the experiment, identification number of inflicted cows and date of diagnosis were recorded by technicians and were attested by veterinarians. The PROC MIXED and PROC GENMOD procedures of SAS were used to analyze metabolic parameters and incidence of health disorders, respectively.

Results: Cows with a 42 and 56 d DP significantly gained more body condition than those with a 35 d DP (-0.002, +0.134, +0.137 for 35, 42 and 56 d DP groups, respectively; P < 0.05). However, the postpartum BCS didn’t change substantially between the groups (-0.29, -0.32 and -0.51 for 35, 42 and 56 d DP groups, respectively). Apparently, the shortest DP, didn’t provide enough time to replenish the body reserves during the DP. However, cows in the 35 d DP group showed a tendency to have higher values of BCS in comparison with those in 56 d DP group at 15 weeks of lactation. This may be attributed to an improved energy balance of cows with a 35 d DP. SP and TP groups had different values of prepartum NEFA
concentration (349.0 ± 30.3 vs. 422.4 ± 25.9 Meq/l for SP and TP groups respectively, *P* < 0.05). For prepartum NEFA no differences were observed between SM and TM treatments as well (375.3 ± 21.0 vs. 324 ± 34.4 mM for SM and TM, respectively). Subsequently, cows in TP group had higher levels of serum NEFA than did the cows in SP group (419.2 ± 25.1 vs. 348.5 ± 30.0 mM for TP and SP groups respectively, *P* < 0.05). Less nutritional stress, more stable rumen flora and less capability of milk production are possible explanations for having lower levels of both pre and postcalving serum NEFA concentrations in SP compared with the TP groups. As expected, cows in SM and SP groups showed substantial differences for the serum triglyceride concentration during late pregnancy (27.3 ± 2.30 and 38.7 ± 3.30 mg/dl for SM and SP d DP, respectively, *P* < 0.01) and during early lactation (26.38 ± 1.96 and 34.85 ± 3.03 mg/dl for SM and SP d DP, respectively, *P* < 0.05). This difference may be attributed to a higher β-oxidation capacity of liver in cows of the SP group. No significant differences were observed in prepartum and postpartum glucose levels across the treatments (72.45 ± 1.35, 72.59 ± 1.46 and 74.35 ± 1.58 mg/dl in prepartum and 80.17 ± 3.2, 74.6 ± 2.9 and 78.35 ± 2.9 mg/dl in postpartum, respectively for cows in 35, 42 and 56 d DP). Likewise, serum insulin (10.67 ± 1.99, 11.61 ± 2.63 and 10.14 ± 2.26 mg/dl, respectively for cows in 35, 42 and 56 d DP) and IGF-I (178.2 ± 20.9, 157.2 ± 19.3 and 136.4 ± 19.3 mg/dl, respectively for cows in 35, 42 and 56 d DP) concentrations were consistent among treatments. Postpartum serum IGF-I concentration tended to be greater for cows on 35 d DP treatment compared with cows in 56 d DP treatment (*P* < 0.1). More improved energy balance, higher plasma glucose and IGF-I concentrations could be an explanation for above results². The incidence of a restricted number of typical postpartum disorders was similar between treatments.

**Conclusions:** In conclusion, regardless of parity, cows with a 35 d DP experienced an improved energy balance or less negative energy balance than the other cows (42 and 56 d DP cows). Assigning dairy cows to a short DP (35 or 42 d) did reveal any change in incidence rate of studied health disorders compared with that of traditional (56d) DP.

**References**

Effect of short dry periods on milk production, composition and reproductive parameters in Holstein cows

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Introduction: It is well known that the dry period (DP) is necessary for mammary gland involution of dairy cattle⁴. Reduction of the DP is beneficial if shortening the DP doesn’t affect the next lactation milk yield. We hypothesize that shortening the DP could be a good management strategy to reduce the diet stress induced by the change in feeding frequency during the dry period. The objective of this study was to determine to what extent the DP length would affect milk production, milk composition and nominal reproductive performance of high-producing dairy cows. Cows were subjected to three different DP treatments: 1) a traditional DP (56d), 2) an intermediate DP (42d) and 3) a short DP (35 d).

Materials and Methods: One hundred and twenty two Holstein cows were selected for this experiment. Cows were assigned randomly to one of 6 treatments as following: traditional DP (56d) for primiparous (TP) and multiparous (TM) cows, intermediate DP (42d) for primiparous (IP) and multiparous (IM) cows and short DP (35 d) for primiparous (SP) and multiparous (SM) cows. Actual DP lengths for respective treatments were 56 ± 5.1d, 42 ± 2.1d & 35 ± 2.7d. Weekly collected milk samples (50 ml) at three consecutive milking (0500, 1300 and 2100 h) were analyzed for fat, protein and SCC. Daily milk production by all cows was recorded through 210 d of lactation. Reproductive parameters were obtained within 4 month after parturition for all cows. The PROC MIXED procedure of SAS⁵ was used to analyze milk yield and composition and reproduction parameter data.

Results: There were no significant differences on milk yield among treatments from 8 weeks pre-partum to 30 weeks of lactation. No treatment effect was observed in the average daily milk production of SM and TM groups from 3 to 210 DIM (Table 1). Likewise, no significant differences were revealed, when milk production adjusted for 305 d lactation milk yield (Table 1). The SP and SM groups produced similar amounts of 305 d adjusted milk yield (Table 1). As expected, cows in SP treatment produced lower (P < 0.01) both mean daily and 305 d lactation milk yield than those in TP treatment (Table 1). Subsequently, cows in SP group were less capable in milk production compared with those in SM group (P < 0.001). Our results, supports the contention that primiparous cows are very susceptible to short DP (35 d DP). Delay in mammary growth, depression of mammary gland performance or combination of both could be reasons for sensitivity of primiparous cows subjected to short DP. This also could be attributed to less differentiated cells in mammary gland at parturition time gaining their productive capacity as lactation cycle approaches. Our results are in agreement with the observations of other studies¹,². As shown in table 1, milk fat percentage and yield were similar across experimental treatments. Likewise, no significant differences were observed in milk protein percentage among treatments (Table 1). However, there was significant difference (P < 0.05) on milk protein yields of 35 and 56 d DP groups (1.06 ± 0.07 and 1.24 ± 0.07 kg/d, respectively for cows in 35 and 56 d DP) which may be explained by less capability of cows assigned to 35 d DP. Cows in IM group had
substantially greater levels of milk SCC compared with those in SM and TM groups (Table 1). We assumed this elevated levels of SCC in IM group bring about by increased nutritional stress during dry period. Cows in SM treatment had greater (P < 0.05) pregnancy rate than those in SP treatment (71 vs. 48 % for SM vs. SP groups). IP group showed a lengthened (P < 0.05) open days (121 d) compared with TP (85 d) group. Comparison of other treatments revealed a consistency in open days data. Days to first service which ranged from 46 to 57 d were similar among treatments. There was a significant difference between cows in SM and IM groups for services per conception (2.0 and 3.0 AI/Conception for SM and IM groups, respectively; P < 0.05), with cows in SP, TP, IP and TM being intermediate. First service conception was greater significantly (P < 0.05) in SM group cows (53 %) than IP group cows (19 %). Concurrent to our results, Gumen et al.\(^3\) reported similar results for some reproductive parameters comparing 28 and 56 d DP.

**Conclusions:** In conclusion, primiparous cows subjected to a 35 d DP, showed considerable milk loss in the next lactation. Shortening the DP had no effect on milk production capacity in multiparous cows. Except with reduced milk protein yield in primiparous cows; shortening the DP to 35 d had no considerable effect on milk compositions. Regardless of parity, cows given 42 d DP revealed a decreased reproductive performance.

**References:**

**Table 1.** Least square means of milk production and composition of cows given different dry periods lengths. SP group are less productive than other groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TM</th>
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<th>IM</th>
<th>IP</th>
<th>SM</th>
<th>SP</th>
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<td>210 DIM milk yield</td>
<td>35.18 (^b)</td>
<td>37.12</td>
<td>34 (^a)</td>
<td>35.1 (^b)</td>
<td>35.42 (^b)</td>
<td>33.74 (^a)</td>
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<td>305 d milk yield</td>
<td>10849 (^ab)</td>
<td>11918 (^b)</td>
<td>10264 (^a)</td>
<td>10656 (^a)</td>
<td>11202 (^ab)</td>
<td>10564 (^a)</td>
</tr>
<tr>
<td>Milk protein yield (kg/d)</td>
<td>1.19 (^ab)</td>
<td>1.29 (^b)</td>
<td>1.00 (^ab)</td>
<td>1.14 (^ab)</td>
<td>1.11 (^ab)</td>
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<tr>
<td>Milk protein (%)</td>
<td>3.19</td>
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<td>3.34</td>
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<tr>
<td>Milk fat (%)</td>
<td>3.79</td>
<td>3.43</td>
<td>3.53</td>
<td>3.68</td>
<td>3.96</td>
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<td>Milk fat yield (kg/d)</td>
<td>1.43</td>
<td>1.29</td>
<td>1.18</td>
<td>1.27</td>
<td>1.45</td>
<td>1.23</td>
</tr>
<tr>
<td>Adjusted SCC (×1000)</td>
<td>320.5 (^a)</td>
<td>476.6 (^ab)</td>
<td>777.7 (^b)</td>
<td>272.9 (^a)</td>
<td>371.5 (^a)</td>
<td>279.8 (^a)</td>
</tr>
</tbody>
</table>
P037 IL-8 concentrations in milk of non-infected and naturally infected Holstein heifers with subclinical CNS mastitis postpartum

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²Institute for Organic Farming, Agricultural Research and Education Centre, Raumberg-Gumpenstein, Austria  
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Introduction: The innate immune system represents the first line of active defense against infection. Extensive neutrophil recruitment into the mammary gland is a hallmark of the early inflammatory response to mammary infection. PMN and mammary tissue containing these infiltrating immune cells produce a range of proinflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-8. High levels of IL-8 can be seen after experimentally induced E. coli infections in cattle and S. epidermidis infections in sheep, whereas S. aureus intramammary infection fails to elicit detectable IL-8. Because the outcome of intramammary infections might correspond with the production of IL-8, we investigated the production of IL-8 in milk of heifers with and without metabolic disorders after parturition to examine its association with occurrence of natural intramammary CNS infections.

Materials and Methods: The study was conducted on a Danish dairy farm with an automatic milking system. Forty heifers calving within a period of 6 months were examined during the first five weeks after calving. Quarter milk samples were collected at weekly intervals. Additionally in the second and 5th week, blood and urine samples were collected. Milk samples were collected aseptically in sterile containers after first performing the California mastitis test (CMT) on each quarter. At each sampling the heifers were clinically examined and feed intake and rectal temperature were recorded.

A loopful (0.01 ml) of each milk was inoculated onto Columbia blood agar and incubated aerobically at 37 °C for 24-48 h. A quarter was considered to be infected with coagulase negative staphylococci if CNS were isolated in at least 2 samples. A quarter was considered as not infected if all 5 samples were bacteriologically negative. For analysis of interleukin 8, milk samples were centrifuged (2500 g, 20 min, 4 °C) and the fat- and cell-free milk fraction was collected. These samples were then frozen at -30 °C until assayed. The concentrations of IL-8 were determined by ELISA using monoclonal antibodies to recombinant ovine cytokine, which were a kind gift of Peter McWaters, CSIRO Livestock Industries, Geelong, Australia.

For diagnosing the metabolic disorders (rumen acidosis and ketosis), catheter urine was checked for ketonuria using ketostix strips. Urine samples were stored at -20°C until assayed for Net Acid Base Excretion (NABE). BHB, GGT, GLDH, AST and TBIL were determined from frozen serum samples.

Results: In total results from 670 quarters were included in the study. 526 quarters were bacteriologically negative and 107 quarters were defined as CNS infected. IL-8 was detectable in milk of 322 quarters. Mean values are shown in table 1. No significant differences in IL-8 concentrations between healthy and CNS infected quarters were observed. Additional information about the metabolic status was available for 267 quarters, from which IL-8 was detected in 126 samples. No correlation between the occurrence of metabolic disorders and CNS infections was seen. IL-8 concentrations were similar in healthy and metabolic disordered groups. CNS infections were associated with a slightly elevated SCC in 46 quarters (42.9 %).

Conclusion: In contrast to experimental CNS infections in sheep, natural occurring CNS infections in this study were not associated with elevated IL-8 levels in milk of heifers.
Metabolic disorders did not influence IL-8 concentrations. Absence of elevated IL-8 concentrations in CNS infections is in accord with the absence or low levels of IL-8 seen in milk during S. aureus mastitis in cows. A poor innate host response to CNS could contribute to the high prevalence of CNS infection. Poor stimulation of IL-8 may be due to cell wall components of CNS such as glycerol teichoic acid.

References:

Table 1: IL-8 concentrations (pg/ml; natural logarithm) in milk of non infected and infected quarters from heifers postpartum.

<table>
<thead>
<tr>
<th>Infection status</th>
<th>Number</th>
<th>Mean (pg/ml)</th>
<th>Se (pg/ml)</th>
<th>Min (pg/ml)</th>
<th>Max (pg/ml)</th>
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<td></td>
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<td></td>
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<td>39</td>
<td>9.95</td>
<td>0.14</td>
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<td>9.55</td>
<td>0.16</td>
<td>7.03</td>
<td>11.01</td>
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</table>

Table 1: IL-8 concentrations (pg/ml; natural logarithm) in milk of non infected and infected quarters from heifers postpartum.
**P038 Peptibolomics of the cows’ udder: peptide profiling of the teat canal**

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**Introduction:** Peptibolomics is a new interdisciplinary area in the -omics family, encompassing the specific study of functional peptides available in vivo. Functional means not only a biologically relevant effect or property, but also potentially serving directly or indirectly a diagnostic or therapeutic purpose. The reason for this new word is to avoid any confusion with peptidomics, i.e. one of the major proteomics approaches in which protein samples are proteolytically digested in vitro using one or a combination of proteases and the resulting peptide-mixture analysed to obtain information of the protein [1]. Innate immunity plays a vital role in the protection of the bovine mammary gland against mastitis. Until recently, the migration of effector cells such as neutrophils and monocytes into the mammary gland was thought to provide the only defence against invading pathogens. However, mammary epithelial cells may also play an important role in the immune response, contributing to the innate defence of the mammary tissue through secretion of antimicrobial peptides and attraction of circulating immune effector cells [2].

This research is part of the comprehensive udder defense-enhancing investigations, with emphasis to cow mastitis, as model for our study of the innate immune system. The following overall major objective of our peptibolomics research has been clearly identified: What is the role of peptides in udder-diseases? To pursue this objective, the proposed strategy is to compare the peptide-profiling of the teat canal fluid with the teat canal epithelium and the cistern mucosa of the cow udder.

**Material and Methods:**

**Sample preparation**

Several extraction solvents were tested:
- Acidified methanol (MeOH/H₂O/HCOOH 90:9:1 V/V/V)
- Tris buffer pH 7.4 containing EDTA, Triton-X and protease inhibitor cocktail
- Chaotropic solvents

Samples were diluted or grinded with extraction solvent and centrifuged. Supernatant liquid was lyophilized and dissolved in mobile phase before analysis. Protein-elimination steps are under investigation.

**LC-MS analysis**

**Conditions:**
- Column: Everest™ C₁₈ (5 µm, 300 Å, 2.1 x 250 mm) + guard column (30°C); coupled with SCX and desalting precolumns
- Mobile Phase: AcN/H₂O gradient with 0.1% m/V HCOOH
- Flow Rate: 0.2 mL/min
- UV detection: λ = 215 nm
- MS detection: electrospray ionization (ESI) ion trap
QC:
- Rational choice of internal standard for qualitative calibration and quantification.
- System suitability tests with human defensins.

Results: Some typical chromatograms from the pilot extraction experiments (on Vydac C18 5 μm 4.6 x 250 mm column) are presented hereunder in Figure 1. The upper chromatograms are the total ion MS chromatograms, while UV detection gave the lowest chromatograms.

Conclusions: The objective of the current study is to compare the peptide-profiling of the teat canal fluid with the teat canal epithelium, and the cistern mucosa of the cow udder. Pilot analytical experiments showed different profiles between the different samples. Investigations are on-going to optimize the sample treatment, analytics and identification of the peptides observed.

References:
Thyroid hormones and cortisol in lactating cows after intramammary LPS administration or Escherichia coli administration.

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**Introduction:** Susceptibility to coliform mastitis is highest around parturition and during early lactation\textsuperscript{3}. During exponential growth and the destruction of E. coli, endotoxins (or lipopolysaccharides, LPS) are released in great amounts. LPS administration induces the same clinical signs as E. coli mastitis and mediates the release of inflammatory cytokines\textsuperscript{4}. The purpose of this study was to compare the effects on endocrine changes using two strategies (i.e. challenge with E. coli and LPS) to induce mastitis.

**Material and Methods:** Twelve healthy East Flemish Red Pied cows were involved in the experiment. Only cows with a milk somatic cell count (SCC) lower than 100,000 cells/ml and free of major pathogens were accepted in the study. Six cows were experimentally infected with \(1 \times 10^4\) cfu/quarter of E. coli strain P4:O32 (A. Hill, Compton, United Kingdom) while the other six cows were infused with 500 µg/quarter of endotoxin (LPS) from E. coli strain O111:B4, both in the left front and rear quarters. To allow adaptation to new environmental conditions, the cows were transferred in individual tie-stalls one week before infection with E. coli and LPS administration. They were fed with approximately 8 kg of concentrates, hay and water ad libitum. In the E. coli and LPS study, blood samples were collected daily, 1 hour after morning milking (i.e. 1.5 hours after the morning meal) on day -1, +1, +2, +3, +6 and +9. On day 0, blood samples were collected immediately before infection (0 hours) and every 2 hours from 2 up to 14 hours after inoculation. Plasma levels of cortisol were analysed using the methods of Blum \textit{et al.}\textsuperscript{2}; T	extsubscript{4}, T	extsubscript{3} and rT	extsubscript{3} were analysed using the methods described by Bertoni \textit{et al.}\textsuperscript{1}. Data were analyzed with a two way ANOVA, including group and time in the model, using the statistical analysis program package Statistix (Analytical Software, Tallahassee, FL, USA).

**Results:** The plasma T	extsubscript{3} concentration following intramammary challenge in both the LPS and the E. coli groups increased, likely justified by feed intake and lighting effects (our unpublished results). The values of T	extsubscript{3} after LPS administration show a peak after 4 hours post inoculation (hpi) and returned to baseline values 10 hpi. The increase of T	extsubscript{3} was more pronounced after E. coli infection, showing a peak value 12 hpi. Baseline values were again obtained 24 hpi. The T	extsubscript{4} concentration peaked in both groups 8 hpi but this increase was not significant. The values returned to pre-challenge levels 12 hpi in the LPS group and 24 hpi in the E. coli group. The rT	extsubscript{3} profile showed a rise in both groups, values increasing approximately 10 hpi followed by a peak 14 hpi in the LPS group and 24 hpi in the E. coli group. Baselines were obtained 24 hpi in the LPS group and 48 hpi in the E. coli group. In the LPS group, plasma cortisol levels increased from 2 hpi, reached a peak value at 4 hpi and returned to a normal level 14 hpi. In the E. coli group, a biphasic increase of plasma cortisol was observed with a first moderate peak at 2 hpi and a second pronounced peak at 8 to 10 hpi. Normal plasma cortisol levels were observed after 48 hpi.
Conclusions: It is clear that intramammary infusion or release of LPS to cows induced a release of cortisol and rT₃ in blood plasma. Moreover, and as expected, the changes in concentration of these parameters were induced faster after i.mam. LPS then compared with E. coli administration. The production and i.mam. release of inflammatory mediators is faster during LPS mastitis because LPS can directly activate macrophages and/or epithelium in the mammary gland to release inflammatory cytokines through binding to the CD-14 receptor. During E. coli mastitis, LPS first has to be released by bacteria before cells can be activated, so the onset of the changes occurs later. However, its effects appeared more pronounced than LPS infusion because cortisol remained higher for a longer time.

References:

Figure 1: Mean changes in T₃, T₄, rT₃ and cortisol in blood plasma of healthy cows after i.mam. E. coli LPS (500 µg/quarter; dark areas) or i.m. infection with E. coli (1*10⁴ cfu/quarter; hatched areas).
**P040 Effects of myramyldipeptide and lipopolysaccharide on CD44 expression on macrophages during the resolution of bovine mammary gland inflammatory response**

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**Introduction:** CD44 may be induced as a result of the signals generated following association of lipopolysaccharide (LPS) with the LBP, and consequential binding of the LPS-LBP complex with the CD14-TLR-4 complex (3). Expression of CD44 may be induced by LPS in macrophages (2). CD44 contributed to the ingestion and clearance of apoptotic cells (6, 1). The resolution of inflammatory response to LPS and muramyldipeptide (MDP) is accompanied by the increased level of apoptotic neutrophils (4, 5). The data mentioned above gives rise to the question: is the resolution of the inflammatory response induced by LPS and MDP accompanied by an increase in CD44 expression on macrophages?

**Materials and Methods:** The experiments were carried out on forty mammary glands of ten virgin, clinically healthy Holstein x Bohemian Red Pied crossbred heifers aged 15 to 20 months. We used intramammary instillation of 20 mL of PBS (control), 500 µg of a synthetic MDP dissolved in 20 mL PBS and 5 µg of LPS in 20 mL of PBS. The cell suspensions were obtained by lavages of the mammary gland at appropriated time-points. The expression of CD44 was determined in flow cytometry during the inflammatory response induced by LPS and MDP in five time-points (24, 48, 72 and 168 hours respectively). Mouse anti-bovine CD44 diluted 1:50 and FITC labelled IgG3 diluted 1:100 were used as the primary and the secondary antibodies, respectively.

**Results:** It was observed that the population of macrophages included two types of the cells: non-vacuolised (NMAC) and vacuolised (VMAC) cells. NMAC (monocyte-like cells) were dominant during the early stage of the inflammatory reaction. VMAC contained phagocytosed apoptotic neutrophils in various stages of digestion. These cells were dominant during resolution (particularly at the last time point - 168 hours).

Non-vacuolised macrophages
Intramammary instillation of MDP and LPS resulted in significant increase of the total count of CD44+NMAC after 24 hours (MDP P<0.01 and LPS P<0.05) in contrast to PBS (Fig. 1). During resolution of the inflammatory response it was observed gradual decrease of total count CD44+NMAC, however the difference to PBS remain statistically significant at time point 48 hours and 72 hours after instillation both inducers (MDP: P<0.05; LPS: P<0.05).

Vacuolised macrophages
The lower total count of CD44+VMAC was observed as effect of MDP and LPS at 24 hours after induction (P<0.01) in contrast to PBS (Fig 2). During resolution the total count of CD44+VMAC was increased. The statistically significant differences were observed at 72 hours after LPS (P<0.05) and at 168 hours after MDP.

**Conclusion:** The vacuolisation of macrophages is accompanied a process of resolution of the inflammatory response. Therefore, the total count CD44+NMAC was decreased and total count of CD44+VMAC was increased. It reflects a functional engage of VMAC in clearance of apoptotic neutrophils during the inflammatory resolution.
References:

This study was supported by Czech Science Foundation (GAČR No. 524/03/1531) and by the Ministry of Agriculture of the Czech Republic (MZ 0002716201).

Fig. 1: The total number of CD44 non-vacuolised macrophages.

Fig. 2: The total number of CD44 vacuolised macrophages.
P041 Staphylococcus aureus mastitis in first parity cows on a dairy farm in Hungary

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Introduction: Staphylococcus aureus mastitis is one of the most common diseases in the dairy production. The estimated losses in Hungary are close to 400 Euros per lactation year for each infected cow 2.
The S. aureus caused mastitis is a contagious disease. The infection can happen horizontally and vertically in the herd. In case of horizontal infection the cows are infected mostly during milking 5. By the vertical process is the feeding of contaminated milk and the fly invasion of the farms of capital importance 3, 4.
The goal of the study was to determine the prevalence of S. aureus mastitis in fresh calved first parity cows as part of eradication protocol on a large scale dairy farm in Hungary and compare the strains in the producing and heifer population. The incidence of the infection was 26% in the producing population. The heifer calves received the milk of the mother in the first 5 days after birth, in the following 2 months milk of treated mastitic cows and the high SCC group was fed to the calves. The heifer calves were raised on a separated farm from 6 months age and were brought back to the dairy farm 2 months before the expected calving.

Materials and Methods: Quartermilk samples of the investigated heifers (n=75) were taken 1-5 days after calving. The samples of the producing cows were taken in the previous 16 months, those of the mother cows were taken during the lactation following the birth of the heifers. The samples were streaked on Columbia blood agar, for identification of the bacteria Baird-Parker agar and Staphylase test (Oxid Diagnostic Reagents) were used. The typing of the strains was done via Pulse-Field Gel Electroforesis (PFGE) according to Bannerman 1 by using the contour-clamped homogenous electric field (CHEF-DR II) system (Bio-Rad Laboratories).

Results: The prevalence of S. aureus mastitis in the first calved cows was 21.3%. One of the heifers had 3 infected quarters, 3 had 2 infected quarters and 13 had only 1 S. aureus positive quarter. The samples of the heifers belonged to 6 different PFGE types (A, B, C, F, G, H). The strains of the producing cows belonged to 7 types (A, B, C, D, E, F, G) (Pict. 1). The PFGE investigation showed close relationship (>95% similarity) between the types A, B and C; D and E; F and G. All of the 7 types were >89% similar, only type H showed larger difference. In 6 pairs were possible to compare the types of the heifer and mother. The pathogens were homologue in only 1 pair, in 5 pairs were the S. aureus strains isolated from the heifers different from the strains causing mastitis in the mother (Fig. 1).

Conclusion: The risk of heifers freshen with S. aureus mastitis in a heavily infected herd is high. The source of the infection is not easy to determine, even if a calf get the colostrum of the infected mother, the bacteria received with the contaminated colostrum doesn’t necessarily cause an infection of the calf. Other management related risks like feeding of contaminated milk may play a major role in the spreading of infection among heifers.

References:

Figure 1: PFGE types of heifers and their mothers. ID numbers are the ear tag numbers of calves (3614) and identification number of their mothers (3614 M)

<table>
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<th>ID Nr</th>
<th>PFGE Nr</th>
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<td>3287 M</td>
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</table>

Picture 1: PFGE types: PFGE groups are named from the top to the bottom (A to H). Line on the top showes the similarity of the genoms. NCT C8325 is the control S. aureus strain used.
P042 Subclinical Mastitis Accompanied Ketosis in Cows in Al-Diwaniya Province / Iraq

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Four hundred blood and milk samples were obtained from lactating cows to determine the prevalence & mortality rate of subclinical mastitis and ketosis, as well as the relationship between them.

The results revealed a highest morbidity rate of subclinical mastitis in cows delivered more than three times and those lactating for more than three months, compared with cows delivered three times or less and those lactating for three months or less, respectively.

The results of comparison, according to the number of delivery and the duration of lactation, showed an increase in pH, Na ion & SCC, and a decrease in lactose & SCC, side to side with the increase of delivery numbers and the increase in the duration of lactation, respectively.

The total prevalence of cows with SCK was 59% according to Ross test compared with 65% according to Rothera's test. These results showed a gradual increase of total prevalence of cows with SCK side to side with the increase in times of delivery and a gradual decrease side to side with the increase of the duration of lactation.

The total prevalence of cows with both SCM & SCK was 48%, and was higher in cows delivered more than three times and those lactating for less than one month.
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