The 29th NKVet Symposium: *Mastitis – new knowledge on diagnostics and control on modern dairy farms*

13-14th May 2013, Reykjavik, Iceland

Proceedings
Welcome

Welcome to NKVet symposium of Mastitis – new knowledge on diagnostics and control on modern dairy farms 13 – 14 May 2013. This 29th NKVet symposium is held in Reykjavik, Iceland.

NKVet, The Nordic Committee for Veterinary Scientific Cooperation, was founded in November 1977 with five Nordic Veterinary Associations agreeing on mutual goals and procedures. An agreement was also made with the Nordic Joint Committee for Agricultural Research (NJK) giving economical support for NKVet. NKJ is again acknowledged for their economical contribution also to this symposium.

NKVet aims are to promote veterinary research cooperation between veterinarians and researchers. NKVet functions as a scientific network, to support researchers and post-graduate students. The Nordic countries have much in common and NKVet considers it to be an important task to continue and strengthen the Nordic cooperation in veterinary science.

The symposia have been open to all and the proceedings are always published. Since 1995, the official language of the symposia and the proceedings have been English and are therefore available for participants from outside the Nordic region, in particular the Baltic countries, and facilitated the invitation of experts from countries outside the Nordic countries.

NKVet is administered by a Board consisting of nine members, one from Iceland and two from each of the other Nordic countries. The Board members are nominated among employees at the veterinary universities and national veterinary institutes, and elected by their respective national veterinary associations. The Board meets twice a year, normally in conjunction with the annual symposia.

Mastitis is still the most important production disease on dairy farms. Production loss in combination with treatment costs and premature culling of infected cows constitute the major costs of mastitis. Mastitis research is very active worldwide, making it important to hold symposia to mediate new knowledge. Furthermore, the introduction of new technology, particularly in association with milking, warrants more research and development of new approaches in the control of mastitis. The symposium will address the latest developments in diagnostics and control of mastitis and is aimed for practitioners, consultants and people involved in research in the area.

The NKVet board and organizing committee welcome you to this symposium.

On behalf of NKVet

Þorsteinn Ólafsson
NKvet board

Grétar Hrafn Harðarson
Programme committee

**Program**

**Monday 13th May, 2013**

**9.00-9.15 Opening: Tuula Honkanen-Buzalski, NKVet**

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| Esa Manninen, Finland                      |
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**Session III. Diagnostics**

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| **13.30-13.45**                           |
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| Diagnostic test properties of a Real-time PCR mastitis test of composite milk samples from milk recordings to identify intramammary infections with *Staphylococcus aureus* and *Streptococcus agalactiae*. |
| **13.45-14.00**                           |
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| Diagnostics of intra-mammary bacterial infections – comparison between a PCR assay and culturing. |
| **14.00-14.45 Key note 2**                |
| David Eckersall, UK                       |
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| **14.45-15.00**                           |
| Ann-Kristin Nyman, Sweden                 |
| Associations between cow factors, intra-mammary infections and inflammatory indicators. |
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**Session IV: Comparative aspects**

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| **16.15-16.30**                           |
| Liv Sølverød, Norway                       |
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| Karin Persson Waller, Sweden               |
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### Session V. Epidemiology and Pathogenesis

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### Session VI. Mastitis Control and Therapy

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Abstracts for oral presentations

The modern Nordic dairy farm (Key note)
Olav Østerås, TINE Advisory Service, Postbox 58, N-1431 Ås, Norway olav.osteras@tine.no

The Nordic dairy farming structure is under a fast development. From 1993 to 2012 the number of milk producers is reduced in Denmark from 15,100 to 3,800, Finland from 33,700 to 10,400, Norway from 26,300 to 10,600, Sweden from 18,300 to 5,200. In total the number of dairy producers in all Nordic countries is reduced from 97,500 herds to 30,700 herds within 20 years. A reduction of 68 %. The number of cows is reduced in the same time by only 25 %. At the same time there has been an increase in herd size, most prominent in Denmark with an increase from 48.2 cows per herd in 1993 to 152 in 2011. In Norway and Finland it has increased from 13 cows till 25 and 30 cows, respectively. The milk production per cow-year is also increased from 6,700 or 7,700 in Denmark, Finland and Sweden till now to 9,000 to 9,500 kg energy-corrected milk per cow-year. In Norway this increase started later due to some quota systems, but is now up to 7,300 kg, with an increase as fast as the other Nordic countries. This means that the total milk production has been very stable in all countries. In 2011 the milk production in all countries was 11,540 million tons, which is only 3 % less than in 1993. Denmark has in fact increased their production by 10 % and Iceland with 24 %, and the rest of the countries have a reduction, Finland with 3 %, Norway with 20 % and Sweden with 13 %. This demonstrates a huge structural change in milk production in the Nordic countries.

At the same time there has been a change in the housing system as example from Norway the typical housing system is moving from tie-stall with concrete surface to free-stall with soft bedding. Soft bedding was implemented by regulation in 2006. This has the impact that milk production increases, clinical mastitis is reduced, as well as teat lesions. This is scientifically demonstrated by Ruud et al. (2010), and these results are, as well reflected in the population epidemiological data. The Norwegian herds are, as well regulated by the animal welfare act and regulations which state that tie-stalls for dairy cattle are not allowed as new buildings since 2004 and all dairy cows should be in free-stall after 2024 (or 2034). This is a driving force for new constructions with herd sizes from 35 to 100 cows in Norway. These herds are well fit for an automatic milking system (AMS). As a result of this, 35 % of the stalls in Norway are now free-stalls, and 50 % of the cows are in free-stall. Additionally 40 % of the free-stalls have AMS and 50 % of the cows in free-stalls are milk with AMS. This means that all together 25 % was milked with AMS in 2010, and we expect in 2013 that about 33 % of the Norwegian cows are milked with AMS. Feeding system is also changing, and partial mixed ration as well as total mixed ration is increasing. A parallel situation with free-tall and AMS is more or less present in all the other Nordic countries.

What does this mean for mastitis? We have data illustrating that with larger herds the treatment for clinical mastitis is decreasing, at least in Norway. Comparing herds with 12-17 cows with herds with more than 50 cows the milk yield is increasing from 6,832 to 7,769 per cow-year, the BMSCC is also increasing from 123,000 to 156,000 in geometric mean, however, the treatment of clinical cases are decreasing from 24.5 per 100 cow-years till 15.0. If we compare AMS herds with herds without AMS, within the same herds size (> 50 cows), there is an increase in milk production from 7,414 to 7,851 kg per cow-year. The BMSCC and the mastitis dynamic parameters; as inflammation rate, new inflammation rate and duration are almost the same, however, the incidence of clinical treatment are decreasing from 16.9 to 13.8 cases per 100 cow-years. Comparing AMS herds with non-AMS herds, the odds ratio (OR) for having S.agalactiae is increasing by 3.9, S.dygsagalactiae 1.23, S.uberis 0.48, S.aureus 0.66, CNS 0.93, Cor spp. 0.79 and C.bovis non-significant. AMS is changing the mastitis pathogens.
Udder health in modern dairy farms – associations with management and grouping of cows
Mari Hovinen1, Timo Hurme2, Kristiina Sarjokari1, Marianna Norring1, Leena Seppä-Lassila1, Timo Soveri1
1Department of Production animal medicine, University of Helsinki, Finland
2MTT Agrifood Research Finland, Jokioinen, Finland

Background
Dairy farms are undergoing structural changes towards bigger farms in the Nordic countries. More information about good management practices and grouping of cows is needed to create guidelines for producers. The main objective of this study was to explore the associations between udder health and grouping of cows.

Materials and methods
Data from 82 dairy herds with an average of 126 cows were collected. The herds were visited in winter 2012. Visit included a structured interview about management and grouping of cows, observations of the cows, and measures of the barn. Herd health recordings provided by the Agricultural Data Processing Centre Ltd. (Vantaa, Finland) were gathered. Data were analyzed with mixed models using SAS 9.3 (SAS Institute Inc., Cary, NC).

Results
Median cowSCC of year 2011 was lower in farms with a milking parlour (PM) compared to farms with automatic milking (AM) (159 000 vs. 197 000 cells/ml milk, SD 46 000 vs. 72 000 cells/ml milk, t-test, P<0.001). Of the PM herds, 70% had more than one group for lactating cows compared with 53% of the AM herds. The cows were grouped based on their age, udder health, or on some other reasons, in 66% of PM and 22% of AM farms. Cows with udder health problems were separated into their own group or in treatment pen in 62% of PM and 19% of AM herds. A group for fresh cows existed in 28% of the PM farms.

In the preliminary analyses the outcome variable, ln-transformation of the median cowSCC in 2011, was examined using a linear mixed model with herd as a random effect. The variables of interest were tested against the outcome with the statistically significant background factors (number of measurements of SCC during 2011, breed, ECM, parity) included in the model.

According to preliminary analysis cowSCC was associated with (with P<0.20):
- all 82 farms: milking type (AM vs. PM), keeping heifers with dry or lactating cows before calving, management of liners after milking of each cow, proportion of calves suckling each other
- 32 AM farms: proportion of cows having to tolerate overcrowding, having an access to pasture or outside pen, keeping heifers with dry or lactating cows before calving, methods used in detection of mastitis, proportion of severe hock lesions
- 50 PM farms: cleanliness of udder, cleanliness of teats, proportion of calves suckling each other, amount of bedding on the stalls

In future analysis, different outcomes (such as clinical mastitis) will be explored, and the complete models will be presented in the symposium.

Conclusions
SCC was lower in the dairy herds with PM, compared to the herds with AM. Grouping of cows and different strategies in udder health management were associated with SCC, with different significant variables depending on the milking system used.

Acknowledgements
This study was conducted in collaboration with University of Helsinki, MTT Agrifood Research Finland and TTS Work Efficiency Institute, funded by the Ministry of Agriculture and Forestry, the Walter Ehrström Foundation, the Finnish Veterinary Foundation and Valio Ltd. We are indebted to the farms for taking part in the study.
Milking and mastitis (Key note)
Esa Manninen,
Valio Ltd, Farm Services, Helsinki, Finland

Background
Mastitis causing bacteria can enter the udder only through the teat canal. Thus keeping the cow’s teats clean and in good condition is very important in mastitis prevention. Wrong working routines prior, during or after the milking or malfunction of the milking machine increase the risk of mastitis.

Prior to milking
Many scientific research results and practical observations have shown that clean udders and legs are linked to better udder health. Clean cows make it also possible to achieve good milking capacity. Calm and stress free milking is the key to good milking. Handling of the cows should be calm, for fear and pain are the most efficient milk ejection inhibitors. It also means less stress for the milker. “Cows love routine” means that handling of the cows and udder preparation should always be done in the same manner, no matter who is taking care of the cows. This is a big challenge for the farm manager.

Udder preparation
Good milk ejection reflex is the key to fast milking and good udder health. Cleaning of the teats and taking fore strips to a cup is a routine that releases oxytocin. The delay from the first touch of the udder to the attachment of the last teat cup should be 1-2 minutes, depending on the pressure in the udder. Cleaning of the teats is the way to reduce the number of bacteria that may enter the udder. In conventional milking taking fore strips is the way to control the milk and follow-up the udder health. CMT is used if abnormal milk is observed. Automatic milking machine (AMM) cleans most of the teats but does not check if the teats become clean. In AMM milk quality is controlled during milking with different sensors.

Milking machine
The milking machine may risk good udder health in many ways. Right vacuum level at the teat end and well-functioning pulsation are the key elements. The attachment of the cluster should be done so that the air inlet through the milking unit is minimized. Research has shown a connection between large vacuum variation in the milk line and higher cell counts. Also the dimensioning and the slope of the milk line are essential when trying to keep the vacuum stable. The cluster position on the udder must be balanced so that the udder is emptied as evenly as possible. The risk of impacts increases when the first quarter becomes empty, which means that the milking machine may work like a seeding machine for bacteria. Choosing the right liner for the heard is a challenge. Kicking cows and miscoloured or hard teats are warning signs that should lead to actions. Mouth piece opening diameter, mouth piece depth and liner barrel diameter are the key dimensions when looking for better liners and milking. Vacuum measurements during milking help to evaluate the situation. A high correlation between high mouth piece vacuum and high cell counts has been shown. The machine-on time must be as short as possible to minimize the mechanical stress on the teats. Automatic cluster removers are nowadays very common. When the udder preparation and cluster attachment timing are done correctly, can the take-off setting of the remover be much higher than the factory setting, commonly around and above 500 g/min. Too high take off setting may lead to incomplete milking which is a risk for mastitis. The right setting must be found individually on every farm.

After milking
Teat dipping is a very common practice. Teat dip solutions must have skin conditioners to keep the skin healthy and soft. Soft skin is easy to clean, elastic and tolerates better the forces that act on the teats during milking. A common fault in AMMs is that the teat dip sprays do not always hit the teats. Cows should remain standing after milking for about 30 min- 1 hour to give time for the teat canal to close.
Microbiological diagnostics of udder infections (Key note)
Karin Persson Waller
Department of animal health and antimicrobial strategies, National Veterinary Institute (SVA), Uppsala, Sweden
Department of clinical sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Detection of udder infections is important both for treatment decisions and for herd control of mastitis. In most cases of mastitis some type of bacterial udder infection, such as staphylococci, streptococci or coliforms, is involved, but other microorganisms may also cause udder health problems. The distribution of udder pathogens varies between herds, regions and countries. Moreover, the distribution of udder pathogens and the proportion of milk samples with no growth differ between clinical and subclinical mastitis. The proportion of cases with no growth is higher in subclinical cases of mastitis than in acute clinical cases of mastitis.

For identification of udder infections quarter milk sampling and aerobic culturing of milk on blood agar is still the international gold standard. Cow composite samples are, however, sometime used, and PCR-assays for identification of the most common udder pathogens in milk are nowadays frequently used in mastitis laboratories in some countries. PCR-assays are also sometimes used in surveys of bulk milk samples, for example for detection of herds infected with Streptococcus agalactiae, and on cow composite samples taken at test-milking. Moreover, analyses of cow composite samples from groups of cows or from bulk milk may be included in udder health investigation of certain mastitis problems. Cow composite samples and PCR-assays have, however, both pros and cons compared to udder quarter samples and culturing. These pros and cons are important to consider when deciding on sampling and analysis methods, as well as when interpreting the results of the analysis. For example, the risk of contamination of the milk sample is an important factor to consider. In many field laboratories of veterinary practitioners culturing of milk samples from clinical cases of mastitis using selective media is, however, still the only diagnostic option.

Microbiological analysis of milk samples usually gives one of the following results (culturing/PCR); no growth/presence of bacteria, growth of specific infection in pure culture/presence of one species, or growth of mixed flora/presence of several bacterial species. It is, however, not certain that the result of the analysis reflects the true status of the milk sample or the udder quarter/cow/bulk tank sampled. It is therefore important to interpret the bacteriological findings carefully in relation to sample type, sampling technique, storage methods, and analysis method. Finally, bacteriological findings must be interpreted in relation to clinical findings, somatic cell counts and previous history of mastitis. The chance for a correct microbiological diagnosis increases markedly if quarter milk samples are taken aseptically, are kept and transported chilled from sampling to laboratory if culturing is used, or taken aseptically in a tube containing a preservative (e.g. bronopol) if analyzed by PCR-assay.

In the presentation, important aspects of microbiological diagnostics of udder infections will be highlighted. Moreover, the introduction of new culture-dependent and culture-independent methods for microbial identification, such as matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) for fast species differentiation (including different coagulase-negative staphylococci) instead of biochemical testing, and studies on the microbiota of milk using metagenomics, will also be discussed.
Real-time polymerase chain reaction and conventional culture in bacteriological diagnostics of bovine mastitis – a comparative study
Heidi Hiittö1*, Rauna Riva2, Tiina Autio2, Tarja Pohjanvirta2, Jani Holopainen3, Satu Pyörälä1, Sinikka Pelkonen2
1Department of production animal medicine, Faculty of veterinary medicine, University of Helsinki, Finland, 2Finnish Food Safety Authority Evira, Finland, 3Thermo Fisher Scientific, Finland, *heidi.hiitio@helsinki.fi

Background
The golden standard method for detection of mastitis pathogens is bacterial culture (BC) [1]. It requires experienced staff and is time consuming, and has been increasingly replaced by PCR tests. Here in Finland, more than 90 % of milk samples are at present analyzed for mastitis pathogens using a commercial PCR test. PCR is fast and sensitive for detecting target bacteria in milk samples [2]. Interpretation of the results is, however, challenging, and requires improvement. For this purpose, we compared the PCR test with conventional BC including identification and enumeration of all bacterial colonies observed.

Materials and methods
A total of 295 quarter milk samples from clinical or subclinical mastitis submitted to the laboratory of Valio Ltd were cultured in the accredited national reference laboratory for mastitis (Evira) and also analyzed using PathoProof Complete-12 PCR assay (ThermoFischer Scientific Ltd) by the manufacturer of the test. For BC, 10 µl of milk was streaked onto whole blood agar plate, and all colonies on the plate were identified and enumerated. The first BC diagnosis (D1) was based on detecting ≥1 cfu of any organism. The second BC diagnosis (D2) was based on the following criteria for a positive sample: *S. aureus* or *Str. agalactiae* ≥1 CFU, *Str. uberis* and *Str. dysgalactiae* ≥3 CFUs, other microbes ≥5 CFUs. In BC, with both interpretations, a sample yielding ≥3 different species was considered contaminated. In PCR, detection of DNA of any target microbe was considered diagnostic. The two methods were also compared in their capability to obtain a microbial diagnosis of mastitis in general: mastitis yes/no. Specificity (Sp) and sensitivity (Se) of the PCR test was calculated using BC as a golden standard, and agreement between the tests was tested with κ-coefficient. For *S. aureus* and CNS the agreement was calculated separately. Correlation between CFUs and genome copy numbers was also calculated for *S. aureus* and CNS.

Results
Out of 295 samples, 252 samples yielded a microbial result by BC and 244 by PCR. Se and Sp of PCR to detect microbial species were 88.3 % and 52.3 % (D1) and 94.2 % and 43.5% (D2), respectively. Agreement between diagnosis “mastitis yes/no” of BC and PCR was κ= 0.25 and 0.31 and for *S. aureus* κ= 0.84 (D1 and D2) and for CNS κ=0.41 (D1) and κ=0.38 (D2) (for all results p < 0.05). Se and Sp of PCR to detect *S. aureus* was 97% and 96%, and to detect CNS 71% and 79% (D1) and 86% and 75% (D2). Correlation between the CFUs and genome copy numbers of *S. aureus* was 0.59 and that of CNS 0.38 (p < 0.05).

Conclusions
This is the first study where PCR assay was compared with BC where all bacterial colonies from the samples were identified and enumerated. PCR test was found to be highly sensitive, but the specificity was low. The best performance for PCR was obtained for *S. aureus*, with probability of 88 % (positive predictive value) of the samples positive by PCR being diagnosed positive also by BC. In contrast, only 41 % of the PCR positive samples for CNS would have been diagnosed positive using BC. PCR test has much potential for mastitis diagnostics, but its diagnostic interpretation still needs improvement.

References
[1]. JS Hogan, RN Gonzales, RJ Harmon, SC Nickerson, SP Oliver, JW Pankey, KL Smith: Laboratory handbook on bovine mastitis. NMC, Madison, WI 1999
Background

Danish farmers can order PCR analysis of the routinely taken milk recording samples with the Real-time PathoProof™ Mastitis PCR Assay. The PCR analysis has a high analytical sensitivity (Se) and specificity (Sp), and is mainly used for individual cows pre dry-off or for herd screenings, e.g. in relation to control of contagious mastitis. This study aimed to investigate diagnostic properties of the PCR test for diagnosis of intramammary infections (IMI) with *Streptococcus agalactiae* (*S. agalactiae*) and *Staphylococcus aureus* (*S. aureus*). Because the PCR tests are carried out on non-sterile taken samples, carry-over between cows and false positive cows due to teat canal infections, teat skin infections, colonization and/or contamination may occur. Therefore, we also investigated if pre-sampling procedures and milking order affected the PCR test results.

Material and Methods

PCR tests were done for all cows in 6 problem herds and in 142 pre dry-off cows in 7 herds. Sterile quarter foremilk samples were subjected to bacteriological culturing (BC) in all pre dry-off cows and in 50% randomly selected cows of the problem herds. The PCR test results were recorded as Ct-values. Latent class models (LCA) were used to estimate the sensitivity and specificity at different Ct-value cut-offs. LCA assumes that no perfect test exists and that both tests, PCR and BC, evaluate an underlying, latent disease, in our case an ‘intramammary infection’. Logistic regression models were used to investigate the effect of teat disinfection on *S. aureus* positivity in the 6 problem herds, while a logistic regression with generalized estimating equations was used to investigate the effect of milking order on *S. agalactiae* PCR positivity.

Results and Discussion

For both pathogens, the Se for PCR increased as expected when the Ct-value cut-off increased, whereas the Se for BC decreased with increasing PCR Ct-value cut-off. That changes of the Ct-value cut-off affected the Se of BC could indicate that the underlying disease definition changed from ‘being pathogen positive’ at high Ct-value cut-off to ‘shedding high amounts of pathogen’ at low Ct-value cut-offs. Pre-sampling procedures, defined as disinfection of the teat end and taking a sterile quarter milk sample for BC, reduced the odds for positivity in the PCR test significantly for *S. aureus* and for *S. agalactiae*. These findings may indicate that contamination, teat skin colonization and or teat canal infections increase the risk of false *S. aureus* and *S. agalactiae* positive cows in non-sterile taken samples for PCR. The preliminary results indicated that minor carry-over occurred, resulting in subsequently milked cows having higher odds of being positive for *S. agalactiae* when considering overall pathogen occurrence. At high concentrations of pathogen, no carry-over could be detected, possibly due to the lower number of cows with high pathogen concentrations.

Conclusion

PCR-tests based on composite milk samples from milk recording can be a valuable tool in mastitis control of *S. agalactiae* and *S. aureus*. The choice of Ct-value cut-off depends on the purpose of the sampling, i.e. whether identification of all positive cows or identification of heavily/truly infected cows is of interest. Disinfection of teats prior to attachment of the milking units should be carried out to reduce false positive results. Minor carry-over effects were shown for *S. agalactiae* and could be handled by accounting for milking order, repeated tests of positive cows and by considering other inflammation markers.
Diagonistics of intra-mammary bacterial infections – comparison between a PCR assay and culturing

Ann-Kristin Nyman¹, Ulf Emanuelson², Karin Persson Waller¹,²
¹ Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden
² Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Background
The golden standard for diagnostics of bacterial intra-mammary infection (IMI) is culturing. However, culturing takes time, can be subjective, and be falsely negative if the bacterial count is low. In recent years PCR (Polymerase Chain Reaction) assays have been developed to, in a more objective and quicker way, identify the most common pathogens in milk. PCR assays identify DNA from both living and dead bacteria in the sample, i.e. also bacteria that have already been killed by the cows’ immune system. Moreover, if these assays are used on non-aseptically collected whole udder samples (e.g. milk samples taken at test-milking), where the risk of contamination is high, the results could be difficult to interpret. Though a lot of research has been conducted to validate the PCR assays, the interpretation of their results is still not clear and needs further investigation. The aim of this study was to determine the diagnostic properties of a multiplex real-time PCR assay, when analyzing whole udder samples, compared to culturing of quarter milk samples, and to investigate associations between bacterial findings and test-milking somatic cell counts (SCC).

Material and Methods
Whole udder test-day milk samples were taken according to normal test-milking procedure and analyzed using PathoProof™ Mastitis PCR Assay (Finnzymes Diagnostics). Quarter milk samples were collected aseptically from the foremilk at the same test-milking. The quarter milk samples were analyzed using routine bacteriological culturing at the National Veterinary Institute. Milk samples from 955 cows were analyzed. To estimate the diagnostic properties of the two diagnostic tests latent class analysis was used in absence of a perfect reference test. The herds were divided into two sub-populations depending on breed (Swedish Red or Swedish Holstein) and the Hui-Walter 2-test 2-population model was applied to estimate Se and Sp for the two tests, and to obtain estimates of the prevalence in the two sub-populations. Logistic regression analyses were used to investigate associations between bacterial findings and SCC.

Results
The results showed that the sensitivity of the PCR assay, varying between 69-97% depending on investigated bacteria (Staphylococcus aureus (Sa), Streptococcus dysgalactiae (Srd), Streptococcus uberis (Sru), Streptococcus agalactiae (Sra), Escherichia coli (Ec) and coagulase negative staphylococci (CNS)), was higher than the sensitivity of culturing (varying between 26-83%). The specificity of culturing varied between 88-100%, and was higher than the specificity of the PCR assay (varying between 28-99%). Findings of Sa, Srd, Sru and CNS, using either diagnostic test, was significantly associated with having a SCC ≥ 100 000 cells/ml.

Conclusions
Whole udder samples analyzed using the specified PCR assay can be used for screening of cows with certain IMI. The larger number of samples positive with the PCR assay could be due to contamination (when using whole udder samples) and/or already killed bacteria, but the associations found between a positive test and a high SCC indicate that most findings were due to infections associated with subclinical mastitis. However, neither the PCR assay nor culturing is a perfect test for identification of IMI.

Acknowledgements
The authors gratefully acknowledge the financial support from the Swedish Farmer’s Foundation for Agricultural Research (Stockholm, Sweden).
The diagnosis of bovine mastitis by examination of milk has, to a large extent, relied on measurement in laboratories of somatic cell count with the California Mastitis Test being a qualitative on farm test. Alternative biomarkers in milk of mastitis have been proposed such as NAGase activity, lactate levels, pH and electrolyte analysis but none have become universally accepted as a replacement test for this economically important condition. Proteins in milk have also been proposed as biomarkers of disease, notably with the acute phase proteins, mammary-associated serum amyloid A3 (M-SAA3) and haptoglobin which are synthesised and secreted in the mammary gland during infection. These proteins are undetectable in milk from healthy udder quarters, while experimental study has shown that their expression can be up-regulated over 80 fold following *Staphylococcus aureus* infection of the mammary gland and in milk their concentration can reach µg/ml levels. The potential for acute phase protein as biomarkers for mastitis resulted from a reductionist approach to investigate pathophysiological responses to disease. An alternative biomarker discovery approach is also possible by utilising recent advances in omic technology such as proteomics, peptidomics and metabolomics. Milk from dairy cows with mastitis has been compared to milk from quarters with mastitis using the proteomic approach of 2 dimension electrophoresis and mass spectrometry and with each advance in technology further proteins can be identified that are up-regulated in mastitis. However, this technology has a limitation in that small peptides of <15 kDa are not retained in the polyacrylamide gel of the second dimension. Recently a capillary electrophoresis and mass spectrometry system has been used to examine change in smaller peptides in milk following clinical mastitis. A panel of peptides which changed in milk during disease were revealed as potential biomarkers of the disease and in addition a modified panel could differentiate between the Gram positive and Gram negative pathogens as bacterial causative agents of the disease. Metabolomic comparison of healthy milk and that from udder quarters with clinical disease has also been undertaken revealing numerous metabolites of milk differ between health and diseased status with potential for diagnosis and monitoring of infection. Advances in omic technologies are providing substantial opportunities for improved diagnosis and for investigation of the pathophysiology of mastitis.
Associations between cow factors, intra-mammary infections and inflammatory indicators

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Background
Correct identification of cows with infectious subclinical mastitis, in a fast and cost-effective way, is highly desirable in order to reduce the risk of spread of udder pathogens in a herd. Measuring the milk somatic cell count (SCC) is the most common tool to achieve this, but analyses of other inflammatory indicators, such as lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAGase) and alkaline phosphatase (AP), are also used. It is important to continuously assess the ability of the inflammatory indicators to find cows with intra-mammary infections (IMI). It is well established that cow factors and IMI contribute to the variation in SCC within and between cows, but how these factors are associated with LDH, NAGase and AP is not as well investigated. The first aim of this study was to investigate if and how different cow factors and IMI are associated with SCC, NAGase, LDH and AP. The second aim was to investigate the ability of the inflammatory indicators to predict if a cow has IMI or not.

Material and Methods
A prospective cohort study was designed, and a total of 25 dairy herds were enrolled in the study and visited twice during the study period. At each visit, quarter milk samples were taken from 20 cows on three consecutive days, the day before the monthly test-milking, the day of the monthly test-milking and the day after the monthly test-milking, for bacteriological culturing. Whole udder test-day milk samples were taken according to normal routines, and analyzed for SCC, LDH, NAGase and AP. A cow was considered IMI-negative if all 12 udder quarter samples were bacteriologically negative, and IMI-positive if one or more udder quarter samples were bacteriologically positive. Associations between the dependent variables SCC, LDH, NAGase and AP, and the independent variables parity, breed, DIM, milk yield, percentage of milk-fat and milk-protein, milk-urea and IMI were investigated using multilevel mixed-effect linear regression models. Milk samples from 976 cows were analyzed, and 522 of those cows were considered IMI-negative.

Results
The results showed that all cow factors investigated were significantly associated with one or more of the inflammatory indicators. Moreover, all inflammatory indicators except AP were significantly associated with IMI status. The cow factors explained approximately 23% of the differences in SCC between cows, but adjusting the SCC for these factors did not improve the ability to predict if a cow had IMI or not. The SCC had better ability to predict if a cow had IMI or not than the other inflammatory indicators.

Conclusions
Cow factors were associated with the inflammatory indicators investigated, but IMI status affected SCC more than the other indicators. Thus, the SCC still seems to be the most useful tool for finding cows with IMI.

Acknowledgements
The authors gratefully acknowledge the financial support from the Swedish Farmer’s Foundation for Agricultural Research (Stockholm, Sweden).
Mastitis in non-bovine dairy species, companion animals and breastfeeding women (Key note)
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The importance attached to mastitis research in past decades stems primarily from the commercial relevance of the disease to the dairy industry, which in Nordic countries and many other parts of the world is based very largely on dairy cows. One could be excused, therefore, for thinking that mastitis is unimportant in other species. In addition to cattle, goats, sheep and water buffalo are commercially important dairy species in many parts of the world, and there is increasing interest in camelids and even equidae as producers of milk for specialist purposes. European small ruminant and buffalo milk production is predominantly associated with the south of the continent, although a number of Nordic sheep and goat milk products are available and dairy buffalo are farmed in several Nordic countries. Historically, products such as Skyr would have been made from sheep's milk as well as cow's, and the increasing market for niche products would suggest that demand for non-bovine milks is likely to increase in the north just as it has done in the south. What is perhaps more relevant in a Nordic context is that animal health and welfare have a high priority for consumers and producers. To regard mastitis as purely a disease of dairy cows might be shortsighted from a commercial point of view, quite apart from potential scientific benefits to be gained from comparative knowledge. There has been a large increase in sheep and goat mastitis research in the last few years, employing approaches that are just as advanced as those used in bovine mastitis. This review will examine what is known about mastitis in non-bovine commercial dairy species, and as far as possible compare this with bovine mastitis. The same species that provide milk are also farmed for meat, beef cattle and sheep being the most relevant in the Nordic context. Regrettably, there is often a tendency to assume that mastitis is restricted to the dairy breeds and does not occur in lactating cows or sheep raising their young. Recent evidence, including some presented at this meeting, shows that this assumption is invalid. The review will also examine problems related to detection and treatment of mastitis in farmed animals kept for meat production. In contrast to sheep and goats, literature on mastitis in other farmed species and in companion animals such as dogs and cats is sparse. That is not to say that the disease does not occur in these species; from anecdotal accounts of veterinary colleagues it is evident that it does occur. Reasons for the relative lack of interest will be examined. Finally, the review will also consider human mastitis. This is a topic that has attracted considerable dispute and disagreement in recent years, such that what might be regarded as “simple” issues (should antibiotics have a place in therapy, for instance) have not achieved consensus. It is evident that mastitis is relatively common amongst breastfeeding mothers, is very painful, can often lead to premature termination of lactation and can be a vector for vertical transmission of diseases including AIDS. Given this, it is somewhat surprising that human mastitis has not received more attention. The review will consider what is known, and propose areas in which progress might be expected in the future, and how knowledge gained in other species might contribute to that progress.
High bulk milk somatic cell counts (BMSCC) has been a challenge in goat dairy production. For the last decades, the mean levels in Norway have been well above 1.000.000 cells/ml. The SCC of a healthy goat is expected to be below 500.000 cells/ml. A national study conducted in 2004 showed a herd prevalence of 88% for Caprin Arthritis-Encephalitis (CAE) based on antibodies in bulk milk samples, and presence of clinical Caseous Lymphadenitis (CLA) in 71% of the milk producing flocks. In the same screening, 40 herds came under restrictions from the authorities due to Paratuberculosis. Former studies have shown CAEV to cause indurative mastitis in addition to the hallmarks of arthritis and neurological dysfunctions. As a result, infected goats frequently show reduced milk yield and increased SCC. The Norwegian Goat Health Services will by the end of 2014 finish a complete sanitation programme for CAE, CLA and Paratuberculosis, rendering all 423 milk producing herds officially free of the three infections.

*Staphylococcus aureus* (*S. aureus*) has been shown through several studies to be the dominating pathogen isolated from clinical mastitis in Norwegian goats. Infections caused by *S. aureus* are well known to have a great impact on SCC levels in goat milk. Infected udders are the main reservoirs of *S. aureus*, but recent studies also show a great occurrence of the bacteria in extramammary bodysites such as the nose and vagina. According to TINE Goat Health Recording System, there is a mastitis treatment level of 2-3% on national basis. As there is a tendency for mastitic goats to be either slaughtered or left untreated, we have reason to believe that these numbers are underestimates of the true incidence level. Control of milking equipment reveals severe dysfunctions too frequently. These may have severe negative effects on udder health.

From 2009 to 2011 8 sanitized herds from Telemark county participated in a study on udder health control. At the beginning of the study, all farms had their milking equipment examined and optimized by a VADIM-test (VAcum Drop In Milking). Each herd committed to collect DHI samples from all lactating goats at least 5 times during lactation. These were analyzed for protein, fat, lactose and SCC. In addition, quarter milk samples were analyzed bacteriologically from all goats before drying off autumn 2009, after kidding winter 2010 and before drying off autumn 2010. A total of 1385 lactations were included. Goats infected with *S. aureus* or *Str. Dysgalactiae* (n=48) were treated with antibiotics at drying off. Prevalence of *S. aureus* infected goats at drying off in 2009 was 7.4%, and 4.8% at drying off in 2010. *S. aureus* prevalence after kidding in 2010 was 5.9%. 85% of the *S. aureus* infected goats treated at drying off did not show infection when sampled after kidding. 7% of the 374 goats that were uninfected at drying off showed infection after kidding. Geometric mean SCC for *S. aureus* infected goats at drying off were 2.842.000 cells/ml, and 547.00cell/ml for the uninfected. Geometric mean at first DHI sampling after kidding was 114.000 cells/ml for the treated goats, 161.000 cells/ml for the non-infected. **Geometric mean SCC for the lactations where S.aureus infection was treated at drying off were 549.000 cells/ml, following lactations 415.000 cells/ml. 52% of the lactations showed SCC lower than 1 million cells/ml in all samples. The prevalence of DHI samples with less than 200.000 cells/ml increased from 24 to 26 from 2009 to 2010.**

Geometric mean BMSCC of the participating herds was 943.000 cells/ml, 839.000 cells/ml, 842.000 cells/ml and 824.000 cells/ml in 2009, 2010, 2011 and 2012, respectively. Most herds did not take action during lactation to correct problems with high SCC goats. Additional udder health control measures during lactation are needed to lower the bulk milk somatic cell count.
Subclinical mastitis and intra-mammary infections in Swedish beef cows

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Background
In beef cows, milk production is the most important factor affecting calf growth. Thus, factors that limit production have negative effects on calf weaning weight. Mastitis is a disease, which reduces milk production and is often associated with bacterial intra-mammary infection (IMI). Studies from UK and USA indicate that subclinical mastitis and/or IMI significantly affects calf weaning weight. The knowledge on subclinical mastitis in Swedish beef cows is, however, very limited.

The aim of the study was to investigate the prevalence of subclinical mastitis, IMI and blind quarters in a small number of beef herds to evaluate the need for more comprehensive studies regarding risk factors and control measures for subclinical mastitis in beef cows.

Materials and Methods
In 10 herds, udder conformation, udder condition, and presence of blind quarters was evaluated and quarter milk samples were taken from 9-12 beef cows per herd approximately one month after calving and close to weaning. The milk samples were analyzed for somatic cell counts (SCC) using CMT and the De Laval Cell Counter, and bacteriology using routine culturing procedures. Coagulase-negative staphylococci (CNS) were differentiated using Maldi-ToF. Subclinical mastitis was defined as SCC ≥200 000 cells/ml at quarter level.

Results
In total, 104 and 90 cows were investigated after calving and at weaning, respectively. Of those, 24% and 10%, respectively, had at least one blind quarter. The proportion of cows with subclinical mastitis or IMI in at least one quarter at the sampling one month after calving was 46% and 40%, respectively. The corresponding numbers at the sampling close to weaning was 58% and 42%, respectively. Intra-mammary infection was found in 13% and 16% of udder quarter samples taken after calving and close to weaning, respectively. CNS and Staphylococcus (S.) aureus were the most common findings. Seven different CNS species were identified, but S. chromogenes was the most common finding (66% of the isolates). S. aureus IMI was found in 14% and 19% of the cows after calving and close to weaning, respectively. Approximately 10% of the cows had S. aureus IMI at both samplings. All parameters varied markedly between herds.

Conclusions
The results indicate that both subclinical mastitis and IMI are relatively common in Swedish beef cows, and that staphylococci are the most common IMI. Whether subclinical mastitis and IMI resulted in a lower weaning weight in calves from affected cows will be evaluated.
Epidemiology and pathogenesis: paradigm shifts in the molecular era (Key note)
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Background
To support the control of mastitis in dairy herds, the main bacterial species associated with mastitis have traditionally been divided into contagious and environmental pathogens. This terminology is primarily epidemiologically orientated and it is useful in the context of population management and prevention of transmission. For management of individual cases of mastitis, a distinction between host-adapted and opportunistic pathogens may be more useful. To some extent, these categories overlap. In recent decades, the use of molecular methods has provided more detailed understanding of the association between species, strains and their transmission and pathogenesis mechanisms.

Materials and Methods
Bacterial isolates were obtained through routine culture of aseptically collected milk samples over the course of a large number of field studies and experimental challenge studies. Isolates were characterized by a wide array of molecular methods, ranging from comparative typing methods such as rapid amplified polymorphic DNA typing or pulsed field gel electrophoresis to definitive typing methods such as multilocus sequence typing or whole genome sequencing.

Results
Results from field studies and challenge studies in numerous countries using a range of molecular characterisation methods have shown that considerable genetic diversity exists within most common mastitis causing bacterial species. Distinct strains or subpopulations may be associated with distinct pathogenesis mechanisms and disease transmission patterns [1]. Both pathogen-associated disease causation mechanisms and herd-associated management characteristics may be contributing factors in the introduction, transmission and persistence of mastitis.

Conclusions
The black-and-white distinction between contagious and environmental pathogens must be replaced with more nuanced shades of grey to capture the heterogeneity within species and to select or develop relevant mastitis control methods (Fig 1).

Acknowledgements
Development of these ideas was supported by Ynte H. Schukken, the team at Quality Milk Production Services, USA, and many colleagues and farmers around the world.

Reference
Infection patterns of *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in early lactation dairy cows

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**Background**
Mastitis is the most common disease among dairy cows and is often caused by bacterial intramammary infections (IMI). Early lactation is a critical period for the dairy cow and a period where such infections are particularly common. It is of special importance to prevent these early lactation IMI since they can have a long-lasting negative influence on milk yield and udder health. The aim was to investigate when IMI caused by *Staphylococcus aureus* (Sa), *Streptococcus dysgalactiae* (Srd) and *Streptococcus uberis* (Sru) occur relative to calving, and if the bacterial findings vary over the year or due to parity.

**Materials and methods**
During one year, quarter milk samples were collected from 740 cows in 13 herds with udder health problems. The first sampling was done before first milking after calving (day 0) and the second four days later (day 4). Samples were sent to the laboratory for culturing and detection of Sa, Srd and Sru.

**Results**
The cow prevalence of Sa was 18.2% at day 0 and 20.0% at day 4. In total, 11.4% of the cows were positive at both samplings. The corresponding numbers for Srd were 14.5%, 9.7% and 7.4%, and for Sru 8.0%, 4.7% and 2.4%, respectively.

There were no differences in bacterial prevalence between parities except for a higher prevalence of Sru in older cows compared to first parity cows at day 0. Some seasonal differences were noted for Sa and Srd. Records of cow somatic cell count (SCC) from the first monthly milk recording after calving were available for 711 cows. Preliminary results indicate that SCC was significantly higher among cows positive for Sa at both samplings compared to cows positive at one sampling or never positive. The same results were seen for Srd. Cows positive for Sru at either one or both samplings had significantly increased SCC compared to cows with no bacterial growth.

**Discussion and conclusion**
It is likely that cows that were bacteriologically positive at both samplings had a true IMI. It is more difficult to interpret the results when cows were positive only at one sampling. Records of SCC indicated that one positive finding of Sa or Srd did not influence SCC, possibly meaning that a single finding was often due to contamination of the sample rather than to a cured (positive only day 0) or emerging IMI (positive only day 4) or to a variable shedding pattern. A single finding of Sru, however, was associated with increased SCC, but due to the small number of Sru-positive cows differences between day 0 and day 4 could not be calculated.

In conclusion, IMI caused by Sa or Srd was common already at day 0 in problem herds in all parity cows, while Sru was less common. Streptococci were more common at day 0 than at day 4. In addition, minor variations due to parity and season were found.
**Introduction**

This study analyses the relationship between positive reactions for *Streptococcus agalactiae* and herd size, milking system and organic or conventional production for the herd surveys in 2009, 2010, 2011 and 2012.

**Material and Methods**

BTM samples from all dairy herds registered in Denmark in December 2009, 2010, 2011 or 2012 were included in the study (n=4258-3759). The samples were collected in August-December each year. In 2009-2011 the samples were tested for 11 mastitis pathogens and staphylococcal beta-lactamase gene blaZ using real-time PCR (PathoProof Mastitis PCR Assay, Thermo Fisher Scientific, Vantaa, Finland). In 2012 the samples were tested using the 16 kit test. Data on herd size and milking system was obtained from the Danish Cattle Database. Data on organic production and a group of herds with conventional production but grazing was also available. A herd that changed milking system or changed between organic and conventional production during a year was excluded from the analysis that year.

Data was analyzed by a generalized mixed model with herd size as a continuous variable and milking system and organic status as categorical variables. PCR test status was used as outcome as a binary variable with a threshold of Ct<40 as positive for *Strep. agalactiae*.

**Results**

Table 1 Prevalence of *Strep. agalactiae* in bulk tank milk in Danish dairy herds

<table>
<thead>
<tr>
<th>Year</th>
<th>No herds</th>
<th>Avg. Herd size</th>
<th>Strep. ag. positive</th>
<th>Herd size &lt;=120</th>
<th>Herd size &gt;120</th>
<th>Not AMS</th>
<th>AMS, Lely</th>
<th>AMS, DeLaval</th>
<th>Organic</th>
<th>Conventional</th>
<th>Conv. grazing</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>4258</td>
<td>135</td>
<td>6.2</td>
<td>4.1</td>
<td>8.2*</td>
<td>5.8</td>
<td>11*</td>
<td>4.8</td>
<td>2.0*</td>
<td>7.5</td>
<td>3.5</td>
</tr>
<tr>
<td>2010</td>
<td>4090</td>
<td>138</td>
<td>6.1</td>
<td>3.1</td>
<td>8.8*</td>
<td>5.8</td>
<td>10.2*</td>
<td>4*</td>
<td>3.0*</td>
<td>6.8</td>
<td>2.0*</td>
</tr>
<tr>
<td>2011</td>
<td>3915</td>
<td>147</td>
<td>5.9</td>
<td>3</td>
<td>8.3*</td>
<td>5.5</td>
<td>9.2*</td>
<td>3.7*</td>
<td>2.5*</td>
<td>6.6</td>
<td>4.0</td>
</tr>
<tr>
<td>2012</td>
<td>3759</td>
<td>157</td>
<td>6.0</td>
<td>3.1</td>
<td>8.2*</td>
<td>5.5</td>
<td>10*</td>
<td>4.8</td>
<td>2.8*</td>
<td>6.5</td>
<td>2.0*</td>
</tr>
</tbody>
</table>

* significance p<0.05 compared to herd<120, milking parlor or conventional

Table 2 Changes in *Strep. agalactiae* status between years

<table>
<thead>
<tr>
<th>Year</th>
<th>Herd size</th>
<th>All herds</th>
<th>Not AMS</th>
<th>AMS, Lely</th>
<th>AMS, DeLaval</th>
<th>Organic</th>
<th>Conventional</th>
<th>Conv. grazing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;=120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;=120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative herd change to positive</td>
<td>1.7</td>
<td>1.3</td>
<td>2.0*</td>
<td>1.8</td>
<td>1.6</td>
<td>1.1*</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Positive herds change to negative</td>
<td>27</td>
<td>46</td>
<td>21*</td>
<td>31</td>
<td>15*</td>
<td>29</td>
<td>26</td>
<td>27</td>
</tr>
</tbody>
</table>

* significance p<0.05 compared to herd<120, milking parlor or conventional

**Discussion**

The analysis showed positive linear correlation between herd size and prevalence of both *Strep. agalactiae* in both 2009, 2010, 2011 and 2012. Significant differences were also found for milking systems. Herd with robotic milking with Delaval VMS had lower prevalence of *Strep. agalactiae* (2010 and 2011) but higher level of *Staph. aureus* in the bulk tank milk than herds with parlor milking whereas herd milked with Lely robots had higher prevalence of *Strep. agalactiae* than other herd groups. Organic herds and grazing herds had significantly lower levels of *Strep. agalactiae* also after correcting for herd size and milking system. Bigger herds showed both higher risk of new infection and lower risk of changing from positive to negative. Herds with Lely robots showed lower chance of changing from positive to negative. Possible mechanisms behind the differences found are now been investigated.
Associations between udder cleft dermatitis and bovine mastitis

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Introduction
Udder cleft dermatitis (UCD) is skin lesions located at the anterior junction between the udder and the abdominal wall, and/or between the front quarters of the udder. In a recent Swedish study, the prevalence of UCD among 1084 cows from 30 dairy herds in a region of Sweden was 18% [1]. The herd prevalence varied between 0% and 39%, with an average of 16%. A possible association between UCD and udder health has been discussed, but very few studies have been published. Therefore, the aim was to study if UCD is associated with bovine mastitis in Swedish dairy herds.

Material and methods
Thirty dairy herds from the county of Östergötland with free stall housing and milking parlor were visited at one milking occasion per herd when every third cow was investigated for UCD. Herd and cow data was collected for risk factor analyses. Udder health parameters investigated at herd level were the mean geometric somatic cell count (SCC) and the incidence of veterinary-treated clinical mastitis (VTCM) during 6 months before the herd visit. Parameters tested at cow level were the cow SCC at the test milking ±15 days from the herd visit and registration of VTCM ±30 days from the herd visit. Associations between UCD and udder health parameters were evaluated using univariable and multivariable regression analyses.

Results
At herd level, significant risk factors in the multivariable analysis were a high proportion of Swedish Red cows (compared to Swedish Holstein cows) and a high production level. In the univariable analyses herds with SCC <200 000 cells/ml had a higher prevalence of UCD than herds with higher SCC, but the prevalence of UCD did not differ between herds with VTCM prevalence <1% or ≥1%. At cow level, the same effects of breed and production level was found in the multivariable analysis. Moreover, older cow were more prone to have UCD than younger cows, and cows with VTCM had a higher prevalence of UCD than cows without VTCM. A significant association between UCD and cow SCC was not found.

Conclusions
Cows with UCD had 3.7 times higher risk for VTCM ±30 days from the UCD diagnosis compared to cows without UCD. Whether UCD is a risk factor for VTCM or vice versa is not clear, but the first option is most likely. It is possible that UCD might be a reservoir for udder pathogens. An association between UCD and SCC was, however, not found. The primary cause of UCD is still unclear, and more research is needed to identify the best ways to prevent the development of the disease.

References
Canada is a very large country geographically, with approximately 12,500 dairy farms spread across 10 provinces spanning more than 5,000 kilometres. Dairy production policy, which controls milk supply, results in a herd size average of 76 cows (provincial range 57-180 cows/herd). Approximately 50% of herds are tiestall and 50% are freestall [1]. In 2013, the Canadian bulk tank somatic cell count limit was lowered from 500,000 to 400,000/mL. This paper reviews recent Canadian mastitis programs and industry recommendations [2].

Recognizing the challenges of mastitis control in such a large and diverse country, a group of 65 scientists from 9 Universities and 2 Federal research centres formed the Canadian Bovine Mastitis Research Network (CBMRN) in 2006. Over the past half dozen years, the program has mobilized resources to help define and answer questions related to mastitis control and milk quality in Canada. Initially, CBMRN sought to define the mastitis situation in Canada. In a random sample of 226 herds, the estimated herd prevalence of *Staph aureus* was 74%, whereas the prevalence of *Strep agalactiae* was <1%. *Staph aureus* was more common in the regions with smaller herd size and tiestalls. Ninety-six percent of herds were using post-milking teat dip, whereas pre-dipping and wearing milking gloves was practiced by 59 and 65% of herds, respectively. Forty-four percent of herds actively tried to segregate *Staph aureus* cows in the milk procedure. Seventy-two percent of herds were doing blanket dry cow therapy and 25% were using internal teat sealants [3]. Regarding clinical mastitis, the mean rate was 23 cases per 100 cow years. *Staph aureus* was the number one cause, especially in smaller, higher SCC herds in eastern Canada, with *E. coli* and no growth predominating in the lower SCC, larger herds from western Canada [4].

Using these preliminary results, CBMRN scientists designed a large cohort study of 91 herds, which were monitored intensively for 2 years. In total, approximately 133,000 milk samples, along with other biological materials and epidemiological information, were collected from cohort herds. Under the nationally standardized procedures of the Canadian Mastitis Laboratory Network, 4 laboratories recovered more than 16,500 pathogens, which have been stored in the Mastitis Pathogen Culture Collection as a legacy to the project. These pathogens, along with coincident herd and cow information, are available to Canadian and international researchers for further investigation. Among clinical mastitis samples (n=7622), *Staph aureus* was the most frequent isolate (13%), with *E. coli* second at 11%. For lactational samples (n~85,000), pre-dry off (n~20,000) and post calving samples (n~20,000), coagulase negative staphylococci (CNS) were predominant (range 4.0-6.5%), with *Staph aureus* being second (range 2.4-3.0%) [4].

The national cohort has provided a rich resource for evaluating intramammary infection definitions, risk factors at the species level and interactions among pathogen types. Wearing gloves during milking showed desirable associations with IMI incidence, elimination, and prevalence of *Staph aureus*. Similarly, adequate teat-end condition and use of pre-milking teat disinfection were associated with lower IMI incidence and prevalence. The initial herd prevalence of *Staph aureus* IMI was positively associated with subsequent IMI incidence [5].

In addition to these epidemiological outcomes, practical studies have evaluated antibiotic use and reduction strategies for dairy herd both for clinical mastitis and dry cow therapy. The continued importance of *Staph aureus* has lead to intensive virulence studies, leading to new vaccine targets and novel potential therapy options. CNS continues to emerge as an important pathogen has lead to ongoing epidemiologic and diagnostic studies.

The CBMRN has not limited its activities solely to research. Both previous and new network-generated knowledge has been disseminated through a knowledge transfer program. This program has directly reached more than 300 dairy veterinarians and 5000 producers. Indirectly, the entire industry has been aggressively targeted through internet and focussed media.

Results from the Danish milk Quality campaign “Our milk - a pure pleasure”, on BTSCC, mastitis treatments, dry cow therapy and dynamics of individual cow cell count from DHI samples.

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Background
In 2010 the Knowledge Center for Agriculture, Cattle launched a milk quality campaign Our Milk – a pure Pleasure. The goals were to reduce BTSCC and the number of initiated mastitis therapy cases. So far the BTSCC has dropped 4% to 221.200 and the number of treatments for mastitis has dropped 16% from 2010 to 2012. These goals have been achieved by direct information to farmers and advisors. The information was focused on: 1) Better milk quality and higher production for better economy. 2) Avoiding or stop of therapy in lactation if culture shows negative result or finding of coagulase negative staphylococci and not to treat cases based on culture or CMT close to calving. 3) Dry cow therapy if contagious mastitis was present in the herd. The introduction of PCR on bulk tank as a part of the surveillance program for Str. agalactiae has also helped advisor to focus on the right advice in each herd.

Material method
In order to focus on better milk quality we started to monitor cows as infected cow if the cow SCC was ≥ 200.000 cell/ml. The status of a cow between two DHI days in lactation or over the dry period was also evaluated on the basis of this threshold. So a new infected cow goes from below to above threshold. A cured cow is defined as dropping below threshold. DHI samples from all Danish dairy herds in the DHI scheme in 2010 -2012 have been evaluated to set a baseline for Danish farmers for benchmarking. Also these dynamic figures have been calculated for herds infected with Str. agalactiae, herds with AMS, and for the different breeds in Denmark. Finally the dry cow periods in 2012 were evaluated on the basis of no therapy, antibiotic therapy, teat sealant or both treatments.

Results
The percentage of infected cows gradually decreased from 30% in 2010 to 28 % in 2012. In 2012 the percentage of infected cows in 1st, 2nd, and older lactations also gradually decreased to 16%, 29% and 41% respectively. Infected heifers at first control day after calving decreased to 21%, new infection rate decreased to 18% and cure rate increased to 32%. In the dry period the results from all cows were new infection rate at 32% and cure rate at 54%. For the non-treated, antibiotic treated, cows treated with teat sealant and for cows with combined therapy in the dry period the new infection rate in 2012 was 31%, 23%, 34%, and 21% and the cure rate was 48%, 64%, 42%, and 72% respectively. Little variation was found in the data from the different breeds with a reduced infection in older cows for Jersey with 38% compared to 41% for DH and more infected heifers at first control day in Jersey with 25% compared to 20% for DH. In the dry period the figures showed no big changes but the use of antibiotic DCT was 31% in Jerseys compared to 24% for DH. Both AMS and Str. agalactiae infection is known to cause higher SCC. For both groups the numbers were quite equal to the overall Danish numbers in 2012. DCT with antibiotics were only used in 29% of the dry periods for Str. agalactiae infected herds, although the use of DCT in these herds is a recommended part of the strategy to reduce mastitis.

Conclusion
Milk quality is getting better in Denmark in combination with lower treatments for mastitis in lactation. A new presentation of the dynamic in cow SCC from one DHI day to the next and over the dry period is introduced to the farmer and advisors in April 2013 and benchmark numbers is calculated so farmers will get more knowledge in which area to improve for even better milk quality.
The Cell Count Emergency
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Background
Swedish farms have the last decade experienced a slow but continuous rise in somatic cell counts (SCC). The main reason for the farmers to pay attention to an increase in SCC is the loss of milk and thus money. Recent studies have estimated a production loss corresponding to 0.9 EUR per 1000 in higher SCC per cow per year. (Hagnestam - Nielsen, 2009; IRL Herd Level Economic Analysis). This means that Swedish farmers, besides the quality payment, lose approximately 25 million EUR due to subclinical mastitis and at least 8 million EUR only because of the last years increase in SCC. It is therefore important that veterinarians as well as other advisors contribute in implementing routines aiming towards a decrease of the Swedish SCC. This will also promote animal welfare and longevity among Swedish milking cows.

Farmers, advisors and veterinarians in Sweden tend to accept that larger new barns with or without robot milking have higher SCC. A multivariate regression analysis of the association between herd level SCC and herd factors also showed that there was a significant correlation (p < 0.001) with elevated herd level SCC in both AMS and large herds in Sweden (Mörk, 2010). Other herd factors that correlated significantly were Holstein breed and low yield, see Table 1. This is the outcome but is it really acceptable? Current literature indicates that few or none unknown risk-factors have emerged in modern farming (Dufour et al 2010; Huijps et al 2009). The increasing SCC levels are instead the result of inconsistent and/or inadequate already known management routines.

<table>
<thead>
<tr>
<th>Herd factor</th>
<th>SCC effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holstein breed vs. Swedish Red</td>
<td>+ 50 000</td>
</tr>
<tr>
<td>&lt; 8,500 kg ECM vs. 9,400 kg ECM</td>
<td>+ 50 000</td>
</tr>
<tr>
<td>&gt; 100 cows vs. 50 cows</td>
<td>+ 30 000</td>
</tr>
<tr>
<td>AMS vs. Tie stall</td>
<td>+ 16 000</td>
</tr>
<tr>
<td>Organic farm vs. nonorganic</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Table 1: Different herd factors effect on SCC, Swedish recorded cows 2009 (Mörk, 2010)

A tool for better communication
In the autumn 2012 an open source web site was established for farmers, advisors and veterinarians. The targeted groups can log on, add present SCC other herd facts and etiology. In return they will receive a brief guidance over actions that primarily should be done. The information presented will be basic but evident, consequent and clear. The idea is that actions for better udder health and lower SCC must be prioritized in an easily understandable way. This will bring higher acceptance and more consistent behavior in the change of routines. A pedagogic stunt for reaching this is a pictured pyramid with ranked areas that need to be secured (Figure 2). To reach the higher levels in udder health you must start thoroughly with the basics. It is in this figure understood that the higher leveled actions will have less or no positive effect if the basic activities are inadequately performed.

Figure 2: Example from the Cell Count Emergency – Mastitis Pyramid, Contagious Bacteria
Modeling the economic consequences of different management strategies against subclinical and clinical mastitis

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Dairy farmers are adopting new technologies for dynamic monitoring of the mastitis status of the individual cow. As these systems provide routinely and more detailed information about subclinical infections, it becomes a management issue, how to optimize mastitis treatment strategies accordingly. The aim of the study was to develop a model for simulating the economic consequences of different management strategies, including indicator based treatment strategies, against subclinical and clinical mastitis. Recent contributions in simulation of mastitis in a dairy herd include differentiation of pathogens, modeling the pathogen specific transmission dynamics, differentiation of clinical and subclinical mastitis, differentiation of the infection and the treatment and differentiation of types of treatment. The contribution of this study was to combine these characteristics in one model and to model detailed treatment criteria according to cow characteristics and alarms for infections. The model includes three different pathogens: Staph. aureus, Strep. agalactiae, and E. coli. Interactions between pathogens, for instance the combined effect of pathogens on mastitis indicators and the effect of medical treatment on more pathogens, are taken into account. In addition, the model simulates interdependencies between herdmates. Replacement depends on availability of pregnant heifers and the extent of contagious transmission depends on the proportion of infected herdmates. The model was implemented in the mechanistic, dynamic and stochastic SimHerd model.

The different treatment strategies included no treatment, treatment of only clinical mastitis, treatment of both clinical and subclinical mastitis, and dry cow treatment. The criteria for treatment of subclinical infections included clinical signs, result from bacteriology or PCR, somatic cell count and lactation stage. Management strategies for prevention of clinical mastitis included vaccination, separation of infected cows, and reduction of contagious transmission.

The model was parameterized according to the literature and new experiments. The new mastitis model was implemented in the SimHerd model and scenarios were simulated for combinations of treatment criteria and management strategies over 20 years. Short- and long term results for the technical and economic consequences of these scenarios in different dairy herds will be presented.
Preliminary results of an investigation of into the use of a polyvalent vaccine in the control of mastitis in UK dairy herds

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Background
Clinical and sub-clinical mastitis remain a major cause of financial loss to the dairy industry and a significant challenge to the dairy producer. There are a large number of treatments and control measures available to the practitioner, but these are often still insufficient to control the disease on farm which in part explains why effective mastitis vaccination has long been the ‘holy grail’ of mastitis control. A polyvalent mastitis vaccine (Startvac©, LABORATORIOS HIPRA SA) recently obtained a European marketing authorisation and is now widely available in Europe. As with other mastitis vaccines the recommended vaccination regime is somewhat onerous and focuses vaccination around calving as the highest time of risk. This has led to the development of a number of more ‘practical’, farmer friendly regimes when using mastitis vaccines such as a rolling quarterly schedule of vaccination. The purpose of the study outlined in this abstract was to investigate whether Startvac©, if used following the label regime or a 3 month rolling vaccination regime, was efficacious in the control of bovine mastitis in dairy cattle managed under UK field conditions.

Materials and Methods
Dairy cows were recruited to the study from commercial UK dairy herds in the south west of the UK. At enrolment, animals, within each herd, were randomly allocated to an unvaccinated ‘control’ group, a ‘label’ or a ‘rolling’ vaccination group. Cows in the ‘rolling’ group were vaccinated on the day of enrolment (day 0), on day 28, day 90 and then every 90 days thereafter until the end of the study. Cows recruited to the ‘label’ group were vaccinated 45 days and 10 days prior to the estimated date of their next calving and 52 days post calving. Herdspersons were trained in clinical mastitis detection, sampling and severity scoring. All cases of clinical mastitis were sampled on each farm from the enrolment of the 1st cow until the end of the study. Treatments and culling was also recorded.

A survival analysis was conducted to investigate the impact of the different vaccination regimes on the incidence rate of clinical mastitis and milk production in early lactation in cows which had been vaccinated at least twice prior to calving. In addition the impact of vaccination on mastitis severity and somatic cell counts was also investigated.

Preliminary Results
3,164 cows were recruited to the study from 7 dairy herds. Data from 1,946 cows was available for analysis, 888, 416 and 642 cows from the ‘control’, ‘label’ and ‘rolling’ groups respectively. Severity data were available from 851 cases of clinical mastitis. The aetiology of clinical mastitis was very diverse encompassing over 30 different species (excluding minor pathogens). Gram -ve pathogens were implicated in 32.2% whilst Staphylococci were implicated in less than 10% of cases. Preliminary analysis failed to demonstrate any difference in the rate of clinical mastitis, somatic cell counts or culling between the treatment groups in the 1st 120 days of lactation. However, clinical mastitis cases occurring in the ‘label’ vaccinated group were significantly less likely to result in clinical signs involving more than changes in the milk than cases in the ‘control’ group (OR 0.58, 95%CI: 0.35-0.98). Clinical mastitis in cows in the ‘rolling’ group was no less severe than in the ‘control’ group. Clinical mastitis severity varied significantly between farms and parities, and was more likely to be severe in cases involving coliform aetiology. Investigation of the impact of vaccination on productivity revealed that cows in the ‘label’ group produced on average 204 litres more milk than cows in the ‘control’ group when other factors such as any effect due to culling, clinical mastitis, yield in the previous lactation, somatic cell count, parity and season of calving had been taken into account. A smaller, but not significant effect (102 litres) was also seen in the ‘rolling’ group.
Conclusions
Whilst this study has failed to demonstrate an impact of vaccination on the incidence rate of clinical mastitis, it has demonstrated the ability of Startvac® to reduce the severity of clinical mastitis in UK dairy herds. Moreover, analysis of production data suggests there is a large and significant increase in yield in cows vaccinated following the licenced vaccination regime that would more than mitigate the costs of vaccination.
Efficacy of immunization with Startvac® in two Swedish herds infected with *Staphylococcus aureus*

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**Background**

*Staphylococcus aureus* (Sa) is the most common bacterial finding in both clinical and subclinical mastitis in Sweden [2,3], thus causing great economic losses for many Swedish milk producers. Beta-lactamase production appears in approximately 3% and 7% of isolates from subclinical and clinical cases respectively [1, 3]. The general approach in controlling the disease is; 1. Identification of carriers, 2. Treating newly infected acute clinical cases with bensylpenicillin, 3. Segregating infected cows from from healthy cows, 4. Treating subclinical cases with DCT at dry off and 5. Culling of chronically infected cows when replacements are available. Important control measures are also good milking technique and hygiene, well-functioning milking machines and the use of iodine teat-dipping. Despite thorough programs many Sa-herds do not succeed in controlling the disease.

The aim of this study was to, in two large Swedish loose housing barns, evaluate the additional effect of a vaccine containing slime-antigen as a complement to long term existing control programs that were regarded as not sufficient by the farmers.

**Material and Methods**

Two relatively large high producing Swedish herds A and B (A=250 SRB cows; B=630 SH cows) participated in the trial. During 12 months all cows in both herds with odd numbers were vaccinated with Startvac® three times round calving in accordance to the protocol advised by the manufacturer. Cows with even numbers were not vaccinated and used as a control group. Milk samples were collected from both clinical and subclinical mastitis cases in both groups during the first four months after parturition. The samples were cultured at the National Veterinary Institute and the results were continuously registered in the Swedish cow data-base. Both herds were connected to the Swedish milk recording system and collected DHI samples for milk yield, fat, protein, urea and somatic cell counts (SCC) every month.

**Preliminary Results**

Since all data on cow level SCC was not available until recently the preliminary results are based on data from 153 cows in the treated and 112 cows in the control group. The results showed no significant differences in incidence of clinical mastitis caused by Sa between the groups in farm A, while a significantly higher incidence of clinical mastitis caused by Sa was detected in the vaccinated group compared to the control group in farm B. In farm A, the cow SCC were significantly lower in the vaccinated group than in the unvaccinated control group at the third test milking after calving. In farm B, however, the SCC tended to be higher in the vaccinated group than in the control group at the same time point.

**Conclusions**

Analyzed data so far show a positive effect of vaccination in farm A. In farm B, however, the udder health was worse in the vaccinated group, which is difficult to interpret. In the final statistical evaluation, other factors such as breed, days in milk and SCC before drying off will also be considered which together with the extended amount of observations might give a clearer picture. Evaluation of all data is in progress and a more well-founded conclusion will be presented at the meeting.

**References**


The effect of Startvac® vaccination on udder health under Icelandic conditions
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Introduction
Traditionally mastitis control is based on preventive measures including good milking technique, clean and dry bedding, feeding according to requirements and identification and appropriate treatment of infected animals. Despite these preventive strategies mastitis is still one of the most important production diseases both from welfare and financial point of view. Much effort with little success has gone into the development of effective vaccines. However in 2009 the European Commission granted a marketing authorization valid throughout the European Union, for Startvac®, a vaccine for cows that contains inactivated bacteria called Escherichia coli and Staphylococcus aureus.

Materials and methods
The effect of Startvac® vaccination was examined on 7 dairy farms with 365 lactating cows in total. The experimental period extended over two years (Oct.-Aug.), the first year being a control year and the second year being a vaccination year where all milking cows were vaccinated. The main vaccination was carried out in Sept. Nov. Feb. and June. with additional vaccinations for heifers and dry cows entering the herds. In Sept. before and after the vaccination period quarter milk samples were collected for bacteriology. Somatic cell counts were studied for individual cows through the dairy recording scheme and for the bulk tank milk (BTM) by weekly sampling. Mastitis cases were recorded both years.

Results
There was a considerable between farm variation but no between year variation for S. aureus and coagulase negative staphylococci (CNS) cultures from quarter samples. E. coli was cultured in a very limited number of samples. Somatic cell counts in BTM were significantly different between farms (P<0.0001) but no between year variation was observed (P=0.41). The mastitis incidence both years was 36% with considerable variation between farms (range: 7%-69%).

Conclusions
In this trial Startvac® vaccination did not have an effect on the parameters studied. Between farm variation for all parameters was considerable, emphasizing the importance of good farm management and strategic mastitis control.

Acknowledgements
The support of The Cattle Development Fund and Hipra is gratefully acknowledged.
Antimicrobials are available by veterinary prescription only in all Nordic countries. Veterinarians are not allowed to make profit by selling drugs. Prudent use guidelines for the treatment of mastitis have been established for decades ago, but their style and degree of details vary between countries. The veterinary committee in the Nordic dairy industries has recently made guidelines for mastitis treatment; these guidelines mainly follow the same principles as the national ones. The basic principle in the Nordic approach is that treatment should be targeted towards the causing agent. The globally common practice in mastitis treatment, “start always with broad-spectrum”, is not used. Before treatment, an aseptic milk sample should always be taken for bacteriological examination. Diagnostic media which allow rapid (overnight) diagnosis are available to be used in clinical mastitis. A commercial PCR-based test for mastitis diagnostics has been launched into market and is now in routine use e.g in Finland. Bacteriological data provide valuable information of the distributions of mastitis causing agents in each herd. Furthermore, when the bacteriological diagnosis is known, treatment can be re-evaluated and targeted towards the specific pathogen.

Bacteriological data on mastitis causing agents and on their susceptibility to antimicrobials are available in the Nordic countries. Mastitis is most commonly caused by coagulase-negative staphylococci (CNS), S. aureus, and streptococcal species. Coliform bacteria cause 10-15% of clinical mastitis. Occurrence of penicillin resistance among staphylococci varies between countries; the proportion of resistant S. aureus isolates has ranged from 5% to 25% and is the lowest in Norway and Sweden. For CNS the figures are somewhat higher. The first choice for treatment of mastitis due to streptococci and penicillin-susceptible staphylococci is benzylpenicillin. The main route of administration of the treatment differs between countries: in Sweden clinical mastitis is mainly treated by the systemic route and in Denmark by the intramammary route. In Norway and Finland combination of both routes may be the most common approach. Use of critically important antimicrobials such as 3rd or 4th generation cephalosporins and fluoroquinolones is avoided in all countries. The selection of products for mastitis treatment varies, but in general is limited compared with other countries of the European Union. Treatment of subclinical mastitis is postponed until drying-off, except in Str. agalactiae outbreaks and in some other defined situations. Dry cow therapy is mostly selective. In the Nordic countries, antimicrobials are not available for the farm personal, but treatment decision and drug selection is made by veterinarians. An exception of this policy is Denmark, where farms with a certain herd health status can have access to antimicrobials.

The present protocols for mastitis treatment in the Nordic countries could be further improved. Duration of routine treatments is longer than in most other countries, which may not be economically justified. Supportive evidence for the superiority of extended treatment exists only for mastitis caused by S. aureus and Str. uberis. Routine use of systemic instead of intramammary treatment increases drug consumption but does not improve cure rates in streptococcal and CNS mastitis. Based on scientific evidence, systemic treatment (in combination with intramammary treatment) can be recommended in S. aureus mastitis and maybe in severe coliform mastitis.

In the Nordic countries the majority of the dairy herds are included in the disease recording systems. Based on these data, the incidence of mastitis is recorded. The adjusted incidences of clinical mastitis vary from 26% (Sweden) to 47% (Denmark). Consumption of different groups of antibiotics for mastitis treatment is monitored by national authorities. Trends and changes in the use patterns of antibiotics can then be followed-up and guidance for the use provided if necessary.
Background

Staphylococcus aureus is the most common contagious mastitis pathogen in many countries [1,2]. It was one of the main targets when antibiotic dry cow therapy (DCT) was initially recommended and implemented. Overall dry cow management significantly impacts udder health, but frequency of adaptation of different drying-off practices, except for DCT, has not been widely reported. The objective of the study was to describe drying-off practice in Ohio dairies and assess their association with a likelihood of a herd being positive for S. aureus.

Materials and methods

A questionnaire was mailed to 780 Ohio dairy producers to survey them regarding general mastitis control and drying off practices in their herds. One to three BTM samples per herd were cultured on selective Baird Barker (BP) agar for detection of S. aureus. Suspect colonies from BP agar were further identified according to National Mastitis Council guidelines.

Results

Response rate to the survey was 49%. Overall, 69% of the responding dairy herds were found positive for S. aureus. Of the responders, 81% reported treating all quarters of all cows with intramammary antibiotics at dry-off, 10% treat only selected cows and 9% do not treat any cows. Thirty percent (30%) of the herds use internal teat sealants (ITS) on all cows and 4% on some. Abrupt cessation of milking is most commonly applied method (in 73% of responders’ herds) to dry cows off, and in 20% of the responding herds milking frequency is reduced and/or ration fed to cows also changed prior to dry-off. None of the drying-off practices (use of ITS, total vs. selective or no dry cow therapy, or method of drying-off) were significantly associated with S. aureus in the BTM, however, they were significantly associated with herd size: larger herds were more likely to treat all cows with antibiotics at dry-off, to use ITS and to dry cows off abruptly than small herds.

Discussion

Prevalence of S. aureus was similar to what has previously been reported from US and Canada [1,2]. Lower probability of S. aureus isolation in BTM has been reported in some studies with total DCT [1], however, none of the drying-off practices were significantly associated with S. aureus prevalence in the current study.

References

Efficacy of enrofloxacin for treatment of acute clinical mastitis caused by *Escherichia coli*

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Background
In Sweden, acute clinical mastitis caused by *Escherichia (E.) coli* is often treated with enrofloxacin, a fluoroquinolone. Treatment of *E. coli*-mastitis has been investigated in many studies, often with contradictive results. Only a few of them have studied enrofloxacin and most of them are experimental trials. To our knowledge only one study on treatment of *E. coli*-mastitis with enrofloxacin is a field trial [1]. No double-blinded trials have been performed to prove the effectiveness of enrofloxacin in the treatment of coliform mastitis.

The aim of this study was to study the effectiveness of enrofloxacin in the treatment of acute clinical mastitis caused by *E. coli* in a clinical placebo trial.

Material and Methods
Dairy cows (n=117) with severe acute clinical mastitis, with suspected *E. coli*, were included in the study. Milk samples were collected from the affected udder quarter for further bacteriology. All cows were treated with a test substance of either enrofloxacin or placebo. Additionally, all cows were treated with meloxicam. A treatment protocol was filled in and sent to the National Veterinary Institute. Cows with growth of *E. coli* were followed up at day 4, 22 and 28 with new protocols and milk sampling (d 22 and 28).

Cows were followed until six months after treatment.

Differences between treatment groups were tested using logistic regression for categorical factors and linear regression for continuous factors.

Results
Fifty-six cows, included in the study, had growth of *E. coli*. Of them, 34 (60.7%) were treated with enrofloxacin (T1) and 22 (39.3%) were treated with placebo (T0). Six (17.6%) of the T1-cows and 3 (13.6%) of the T0-cows died within the first days (p=0.5). The incidence of paresis was higher among the cows that died (p=0.04). At follow-up at day 4, day 22 and day 28 the T1-group had lower proportion of cows with clinical signs, CMT ≥3, and a positive *E.coli*-growth but the differences were not statistically significant (p>0.06). There was a statistically significant difference in milk production between the treatment groups at follow-up at day 22 and day 28. At long term follow-up, there were no significant differences in cow survival or milk production between cows treated with enrofloxacin or placebo. Cows treated with enrofloxacin had a significantly lower somatic cell count until 3 months of follow-up. Cows with severe growth of *E. coli* had a higher risk of dying (0-180 days) and also lower milk production at 5 and 6 months of follow-up.

Conclusion
There was no difference in survival between cows treated with enrofloxacin or placebo; neither on short nor long term follow-ups. Cows treated with enrofloxacin had a higher milk yield at short term follow-up, but not at long term and also lower somatic cell count until 3 months of follow-up. Based on the results of this study, we cannot recommend enrofloxacin as a treatment of severe acute clinical mastitis caused by *E. coli*.

Staphylococcus aureus virulence factors in bovine mastitis
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Background
Staphylococcus aureus (S. aureus) is the most common bacteria causing bovine mastitis in Sweden and many other countries. To improve animal health and reduce economic losses for the farmers, it is essential to reduce the prevalence of mastitis. In previous studies where S. aureus was isolated from acute clinical bovine mastitis in Sweden (N=184), we found that three strains (defined by pulsed-field gel electrophoresis, 100% band homology) accounted for 74 % of the isolates. This suggests that these strains are more prone to spread and cause clinical mastitis (1, Åsa Lundberg personal communication). To be able to identify highly pathogenic strains knowledge about the virulence factors of S. aureus is needed.

Materials and Methods
A subset, consisting of 87 of the previously investigated isolates of S. aureus, was further characterized by a DNA microarray hybridization assay (Identibac Genotyping System). The microarray assay covers 334 sequence targets which correspond to 171 genes and gene variants in S. aureus, most described as virulence genes or as involved in immune evasion. Up to this date only genes coding for staphylococcal enterotoxins (SEs) have been analysed. Fiftyone of the isolates with se genes were further analysed for the ability to produce staphylococcal superantigen toxins (SAgs), including SEs and TSST-1. All strains were grown in a rich bacterial growth medium (Brain heart infusion) and toxin concentrations were analyzed using a quantitative sandwich ELISA.

Results
The prevalence of the se and tsst-1 genes was 75% and 28%, respectively. There were up to 11 different se genes per isolate. In the most prevalent pulsotype all isolates but one carried the egc-cluster, coding for 6 SEs. These isolates also had the genes for agr II. This agr type has previously been reported to be associated with high prevalence of bovine mastitis (2).

Preliminary results show that all isolates possessing one or more toxin genes coding for SEA, SEB, SEC, SED and TSST-1 also produced the corresponding toxin/s/ but at different levels (3). While SEA, SEB and SED were produced at low levels (0-10 ng/ml), the production of SEC and TSST-1 was markedly higher (100-1000 ng/ml).

Conclusions
Genes coding for SEs and TSST-1 are commonly carried by S. aureus isolated from bovine mastitis. The potential role of SAgs in the infection process is unknown. The se and tsst-1 genes are expressed during experimental conditions and SEs and TSST-1 are produced.

References
Multiple-locus variant-repeat assay (MLVA) is a useful tool for molecular epidemiologic analysis of *Streptococcus agalactiae* strains causing bovine mastitis

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Group B streptococci (GBS) were considered a major cause of mastitis in cattle until preventive measures succeeded in controlling the disease in the 1970s and 1980s. During the last 5–6 years an increasing number of cases have been observed in some Scandinavian countries. A total of 187 GBS isolates from mastitis cases were collected from 119 animals in 34 Norwegian farms in the period from April 2007 to November 2010. 133 (71%) of the isolates were from farms with automated milking systems. The strains underwent typing of capsular polysaccharides (CPS) and surface proteins, and were analyzed by multi-locus variable repeat assay (MLVA) to investigate the epidemiological relationship of strains within and between farms. The GBS strains were differentiated into 12 types by CPS and surface protein analysis, with CPS types V (54%) and IV (34%) predominating. MLVA was superior to CPS and protein typing for strain differentiation, resolving the 187 strains into 37 types. In 29 of 34 farms all GBS strains had identical MLVA profiles specific for each farm. However, in one farm represented with 48 isolates, four MLVA variants with differences in one repeat locus were observed during the almost 3-year long collection period. Similar variations were observed at four other farms. This might reflect the stability of repeat loci under in vivo conditions. Farms with automated milking systems were overrepresented in this material. In conclusion, the five-loci MLVA allowed rapid high-resolution genotyping of the bovine GBS strains within and between farms.

**Table 1:** Distribution of capsular polysaccharide types (CPS) and surface protein among all MLVA types and all strains (n=187). One MLVA type had both type IV and non-typable strains (NT).

<table>
<thead>
<tr>
<th>CPS</th>
<th>Protein</th>
<th>No. of strains</th>
<th>No. of MLVA types</th>
<th>No. of farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>Alp2/3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Alp1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>non-typable</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ib</td>
<td>Cα, Cβ</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>Alp2/3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cα, Cβ</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>Alp2/3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>Alp1</td>
<td>64</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>V</td>
<td>R4, Cβ</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Alp2/3</td>
<td>100</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>IX</td>
<td>Cα, Cβ</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-typable</td>
<td>Alp1</td>
<td>2*</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td>187</td>
<td>37</td>
<td>34</td>
</tr>
</tbody>
</table>

* These two strains had an identical MLVA profile as two type IV, Alp1 strains. All four strains were from the same farm.

**Reference**
[1]. A Radtke, T Bruheim, J E Afset, K Bergh: Multiple-locus variant-repeat assay (MLVA) is a useful tool for molecular epidemiologic analysis of *Streptococcus agalactiae* strains causing bovine mastitis *Veterinary Microbiology* 2012, 157: 398-404
Introduction of MALDI-TOF in the routine diagnostics of bovine udder pathogens

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Background
Matrix-Assisted Laser Desorption/Ionization – time of flight (MALDI-TOF) is a coming new technique to identify bacteria. The method is faster, cheaper and can identify more species than conventional biochemical typing methods [1]. The aim of this study was to evaluate if MALDI-TOF can replace traditional tools to identify bovine udder pathogens. We also wanted to study if the new technique can differentiate between the most common coagulase-negative staphylococci (CNS) isolated in association with clinical and subclinical mastitis.

Materials and Methods
In total, 217 isolates were investigated with MALDI-TOF. Thirty-five of the isolates were reference stains from a broad range of animal pathogens. 78 were CNS strains isolated from cases of mastitis and identified with tuf gene sequencing [2]. The remaining isolates consisted of 13 atypical isolates sequenced with 16s rRNA as biochemical typing methods could not identify the species, and 126 isolates (50 Staphylococcus aureus, 23 CNS, 17 Streptococcus dysgalactiae, 14 Streptococcus uberis) from routine-samples from bovine subclinical and clinical mastitis, which were also analyzed using routine biochemical methods. If the initial score was below 2 the samples were reanalyzed. If the reanalyze score remained below 2 an extraction method originating from Bruker was used.

Results
The MALDI-TOF method correctly identified 33 of the 35 reference strains, one of the strains (Bacillus subtilis) was not identified, and an isolate of Staphylococcus pseudintermeius was misidentified as Staphylococcus intermedius.
Seventy-seven of the 78 CNS isolates were correctly identified, and the remaining isolate, Staphylococcus lentis, was identified after extraction.
Eight of the atypical isolates were undoubtedly identified (including all six strains of Staphylococcus aureus) with MALDI-TOF, but an isolate of Nocardia spp. could not be identified at all. Three strains of Arcanobacterium pluranimalium had to be examined using the extraction method to positively identify two out of three strains. A strain of Streptococcus dysgalactiae sp equisimilis could not be identified to the subspecies level. All of the 126 isolates from the routine samples, with the exception of one strain of Staphylococcus conii, were identified with the same result as with biochemical testing.

Conclusion
MALDI-TOF is well suited to replace biochemical testing in routine diagnostics of most udder pathogens from bovine samples. It is also a cheap and easy method to differentiate between the most common CNS found in milk samples from bovine subclinical and clinical mastitis. The new method is now implemented and well functioning at the Department of Bacteriology.

References
The development of lactate dehydrogenase and milk yield during spontaneous Escherichia Coli mastitis in dairy cows
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In dairy cows, E. coli mastitis leads to reduction in milk yield and changed concentration of lactate dehydrogenase (LDH) in the first weeks after onset of the disease. We aimed to examine time-related changes in LDH during E. coli mastitis and to explore/investigate whether these changes can be used to predict changes in milk yield.

Data included 57 cases of spontaneous E. coli mastitis from two Danish Holstein/Jersey herds with free stalls and automatic milking systems (AMS). The presence of E. coli bacteria was confirmed on day 0 and LDH measurements were performed by Herd Navigator (Lattec I/S, Hillerød, Denmark) from day -20 to 28. LDH was measured as the total amount in the milk (milk yield*concentration).

Baseline values for LDH and milk yield was calculated as a mean of samples from day -7 to -3, and the percentage-wise changes were used for the statistical analysis. The percentage-wise changes in milk yield were analyzed as a continuous dependent variable in a mixed model including the repeated-measures statement in PROC MIXED of SAS Enterprise Guide 5.1. Farm number and day were included as fixed effects and the percentage-wise change in LDH as a continuous covariate.

Changes in LDH and day significantly correlated with changes in milk yield (p<0.001). Figure 1 shows the co-development within the two parameters during E. coli mastitis.

**Figure 1**

LDH measurements are used as an early indicator for mastitis by the Herd Navigator® systems. The present results suggest that measurements of LDH are correlated with changes in milk yield during E. coli mastitis.
Intramammary antibiotic usage and antimicrobial susceptibility of beta-lactamase positive S. aureus isolated from clinical mastitis in Estonia 2008-2012

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Background

Different antimicrobials are used for the treatment of clinical mastitis of dairy cows. In Estonia, 18 intramammary product with 10 different antimicrobials are available for the treatment of clinical mastitis in lactating cow. The number of dairy cow in Estonia is ~95000.

Materials and methods

Beta-lactamase positive Staphylococcus aureus isolates (n = 175) were tested for antimicrobial susceptibility by determination of minimum inhibitory concentration (MIC) using microdilution methods (VETMIC™, Uppsala, Sweden). For interpretation of results, the epidemiological cut-off values issued by European Commitee on Antimicrobial Susceptibility Testing were used. Data of wholesale statistics from State Agency of Medicines were used for calculation of amount (kg) of different active substances used for intramammary (IMM) treatments of clinical mastitis.

Results

Annually 90 kg of active substances of antimicrobials was sold for the IMM treatment of lactating dairy cows. The most frequently sold active substances were lincomycin (33.6%), cloxacillin (23.5%), ampicillin (8.2%) and cephalosporins (7.2%). Penicillin was sold only 5.3% of total amount of active compounds.

Table 1. Distribution of MIC of beta-lactamase positive Staphylococcus aureus (n = 175) from clinical mastitis in dairy cows collected during the national antimicrobial monitoring program.

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>Resistance Distribution (%) of MICs (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.06</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>4.6</td>
</tr>
<tr>
<td>Oxacillin+2%NaCl</td>
<td>6.9</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.3</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>13.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.7</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>2.9</td>
</tr>
<tr>
<td>Fucidic acid</td>
<td>7.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>2.3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4.6</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* The proportion of resistant isolates against different antimicrobials are in bold

Conclusion

Clinical mastitis treatment with broad-spectrum antibiotics can lead to high resistance level of S. aureus in Estonia.
Vaccination with a commercial mastitis vaccine Startvac® did not affect the incidence of clinical mastitis in a large dairy herd

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² Department of Production animal medicine, Faculty of Veterinary Medicine, University of Helsinki, Saarentaus, Finland

Background
Mastitis vaccines have been developed for decades, but evidence on their efficacy is still debatable. The only commercial vaccine authorized in the EU (Startvac®, Hipra, Spain) is aimed for herd immunization of dairy cattle to reduce the incidence of subclinical and clinical mastitis, and to reduce the severity of clinical signs of mastitis caused by Staphylococcus aureus, coliforms and coagulase-negative staphylococci. In the USA common core vaccines have been used for decades to prevent coliform mastitis, and in most studies they have been shown to reduce the severity of coliform mastitis. This study was carried out to explore the efficacy of Startvac® in a large dairy herd in Estonia.

Materials and methods
The study herd had 495 lactating cows, with an average milk yield of 9200 kg per year. The herd had problems of mastitis mainly caused by Escherichia coli and Staphylococcus spp. Cows were vaccinated with Startvac® according to manufacturer’s instructions (3 vaccinations: 45 d before the expected parturition, 35 d later i.e. about 10 days before the expected parturition, and 62 d after the second vaccination). In total, 132/216 of the cows in the first lactation and 70/213 of the cows in the second lactation were vaccinated. The rest of the cows served as untreated controls. Cows were selected randomly for the vaccinated group.

The experimental period was from December 2010 to December 2011. Mastitis control program including all important management practices was implemented in the herd. Lactation numbers, calving dates, cases of clinical mastitis and clinical signs of mastitis were recorded. Bacteriological etiology of mastitis was determined using a selective media containing blood-aesculin, McConkey, mannitol-salt and penicillin agars (Mastiidi kiirdiagnostika, Estonian Veterinary and Food Laboratory). The statistical software Stata 10.0 (StataCorp, Texas, USA) was used for chi square test and other statistical analyses.

Results and conclusions
A total of 160 cases of clinical mastitis were diagnosed among 429 dairy cows in first or second lactation. Clinical mastitis was diagnosed in 45% of the vaccinated and in 54% of the non-vaccinated cows in first or second lactation, with no statistical differences between the groups. Occurrence of clinical mastitis between the cows in the first and second lactation and the older cows did not differ. E. coli (n=59) was isolated in milk samples from the affected quarters in 35 vaccinated cows and in 23 non-vaccinated cows. S. aureus and CNS (n= 26) were isolated in 12 vaccinated and 14 non-vaccinated cows. Median days in milk of the cows at the first diagnosis of E. coli mastitis was 33 days in vaccinated and 27 days in non-vaccinated cows; 27% of vaccinated and 20% of non-vaccinated cows had mastitis within the first 3 days post partum. Severity of clinical signs did not differ between the groups. We conclude that vaccination with Startvac® had no impact on clinical mastitis in the study herd. According to the Summary of Product Characteristics, the vaccine is aimed for herd immunization. This may be more important for mastitis caused by contagious pathogens such as S. aureus, but here no effect was seen for any pathogens.
Surveillance of *Streptococcus agalactiae* in Denmark and change from culture to Real time PCR based testing of annual bulk tank milk samples

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Background

*Streptococcus agalactiae* (*Str. agalactiae*) is an increasing problem in Denmark with 5.7% of the dairy herds being included in the Danish *Str. agalactiae* register in 2008 [1]

Materials and Methods

The surveillance program for *Str. agalactiae* in Denmark is based on annual testing of BTM from all dairy herds. From 1964 until 1994 the number of annual testing varied and since 1995 the screening is bases on one annual sampling. BTM samples are collected as part of the routine milk quality program in the trunk. The samples were immediately stored on ice and delivered to Eurofins Steins Laboratorium A/S (Holstebro, Denmark) within 24 h. Until Sept. 2011 a 120 µl milk sample was cultured on blood agar with selective antibiotics and staphylococcus-tox in [1]. Since 2009 all BTM samples have also been tested by Real time PCR PathoProof [2]. Since Sept. 2011 results of the testing have only been based on PCR at a cut-off of Ct-value <40. In spring 2011 information of risk of carry over when sampling in the trunk resulted in retesting of samples taken directly from the tank of 36 herds where the previous herd had an equal or lower Ct value. In 2012 all negative herds with a positive result were controlled for carry over. In herds with risk of carry-over a new sample was taken. Resampling was done in 37 herds directly from the tank. Results from 2009 and 2011 were evaluated for risk of carryover from the previous herd and also on basis of the herd results from other years. In 2009 all herds were tested by culture and PCR of the same milk sample. In 2010 only previous *Str. agalactiae* positive herds and new herd with a Ct value < 40 were tested by culture of the same milk sample that were used for the PCR test. In 2011 herds with a PCR value < 40 for *Str. agalactiae* were tested by culture 3-5 months after the initial PCR sampling based on a new BTM sample from the herd.

Results

Table 1 Results of the annual testing for *Str. agalactiae* in Denmark 2009 – 2012.

<table>
<thead>
<tr>
<th>Year</th>
<th>herds</th>
<th>PCR positive</th>
<th>Carry over PCR</th>
<th>Culture Positive</th>
<th>Carry over Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>4258</td>
<td>263 (6.2%)</td>
<td>63</td>
<td>168 (3.9%)</td>
<td>31</td>
</tr>
<tr>
<td>2010</td>
<td>4090</td>
<td>250 (6.1%)</td>
<td>42</td>
<td>135 (3.3%)</td>
<td>4</td>
</tr>
<tr>
<td>2011</td>
<td>3915</td>
<td>232 (5.9%)</td>
<td>38</td>
<td>158 (4.0%)</td>
<td>7</td>
</tr>
<tr>
<td>2012</td>
<td>3759</td>
<td>228 (6.1%)</td>
<td>34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

Although Danish surveillance program for *Str. agalactiae* has changed to a more sensitive test in Sept. 2011 the former increase in positive herds from 2% in 2000 to around 6% in 2008 has stabilized around 6% from 2009 to 2012.

Reference


The early phase transcriptome of bovine monocyte-derived macrophages infected with *Staphylococcus aureus* in vitro

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2 ARK-Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian EH25 9PS, UK
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Mastitis is the most frequent and costly disease in dairy production. Reduction in the incidence of mastitis will improve farm income, reduce the use of antibiotic drugs in milk production as well as reduce animal distress. Macrophages serve as the first line of innate immune defence against invading pathogens and are critical effectors and regulators of inflammation. In the mammary gland, local recruitment and action of macrophages is a key immunological defence mechanism against infection. We have examined an early phase gene response of bovine macrophages to infection with live *S. aureus*. Genome-wide transcript profiling of blood monocyte-derived macrophages from six Norwegian Red (NRF) heifers infected with live *S. aureus* for 2 and 6 hours *in vitro* was performed. About 420 of the 17 000 genes on the ARK-Genomics bovine cDNA array were differentially regulated at 6 h post infection. Approximately 70% of the responding genes had a known identity (Entrez Gene ID) and were further used for identification of overrepresented pathways and biological functions in the dataset. Several canonical pathways were found, mainly cellular immune response and cytokine signaling pathways, i.e. TREM1 (triggering receptor expressed on myeloid cells 1), IL-10 (interleukin 10) and IL-12 pathways. Among central molecular and cellular functions found were death, survival and movement of immune cells. CD40LG (CD40 ligand) were identified as an upstream regulator, which points toward the CD40 signaling as a central pathway in *S. aureus* induced inflammatory response of bovine macrophages. Moreover, analysis of the subset of differentially regulated genes obtained by comparison with data from genome-wide association mapping in NRF cattle identified peptidoglycan as an upstream regulator, which indicates that this bacterial cell-wall component might be pivotal in macrophage recognition during inflammation.

In this study we have identified several novel candidate pathways and genes likely involved in response to live *S. aureus* infection of bovine macrophages. Our study adds novel insights into the mechanism of cellular immune response during mastitis in dairy cattle.
Influence of bacterial genotype on outcome of clinical Staphylococcus aureus mastitis
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1National Veterinary Institute, Uppsala, Sweden,
2Swedish University of Agricultural Sciences, Uppsala, Sweden

Background
In Sweden, Staphylococcus aureus (Sa) is the most common pathogen causing acute clinical mastitis in dairy cows. In most such cases, antimicrobial therapy is recommended, but the outcome is variable. The reasons for this are not clear, but may be due to differences between Sa genotypes. In a previous study on a limited material three dominating Sa genotypes were found [1], but the influence of bacterial genotype on outcome has not been studied in Sweden. The aim of the study was to gather more information on genotype variation of Sa in Sweden and to investigate if Sa genotype influences the outcome of veterinary-treated clinical mastitis (VTCM).

Materials and methods
Isolates were collected from cases of naturally occurring VTCM in a previous national survey of microbial aetiology of acute clinical mastitis. One isolate per herd (n=184) was genotyped using pulsed-field gel electrophoresis (PFGE), and was classified into pulsotypes (PT), with 100% similarity, and PT clusters (>80 % similarity). When investigating the outcome of VTCM, all cows with more than one infected udder quarter, and cows with mixed infection in one udder quarter were excluded. Outcome measurement used was cow composite milk somatic cell count (SCC) during four months following VTCM. Records were present for 111 cows.

Results
In total, 29 PT were found, and PT PQ (43%), PT TU (22%) and PT E (9%) were most common. The remaining 26 PT were found in 1 to 8 herds each, but the majority (n=17) was only found in one herd. When investigating the outcome of VTCM, the lower level of genetic relatedness (PT clusters) was used. The common PT clusters III and VIII (accounting for 72% of the cases) were compared with less common/rare clusters. The proportion of cows with SCC <200,000 cells/mL at all test-milking from 14 to 120 days after VTCM was higher in the group infected with common clusters (~50%, 95% CI: 37.8-59.7%) than in the group infected with less common/rare clusters (~20%, 95% CI: 4.3-35.7%). The mean SCC during the follow-up period was ~350,000 cells/mL for common clusters, and ~600,000 cells/mL for less common/rare clusters (P=0.002). The same cluster difference in SCC was also found when using a multilevel mixed-effect linear regression model including breed, parity, DIM, ß-lactamase production and milk yield.

Conclusions
In Swedish dairy herds, a few Sa genotypes are widespread, with two genotypes identified in about 65% of clinical cases of Sa mastitis. The long-term outcome of those cases differed between genotypes. Cows infected with the most common genotypes had lower SCC during 120 days after treatment compared to cows infected with less common or rare genotypes.

References
PathoProof KingFisher Duo DNA extraction kit for PathoProof Mastitis PCR Assay
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Thermo Fisher Scientific Oy, Vantaa, Finland

Abstract
The PathoProof™ KingFisher™ Duo DNA extraction kit provides a semi-automated DNA extraction protocol for faster and more convenient sample preparation. The sensitivity and repeatability of the KingFisher Duo method are statistically comparable to the manual PathoProof DNA extraction and to PathoProof KingFisher Flex DNA extraction. The PathoProof KingFisher Duo DNA extraction method purifies samples from PCR inhibitors more efficiently than the manual PathoProof DNA extraction method.

Picture 1. Both total time of extraction and the hands-on time were significantly shorter with KingFisher Duo DNA extraction compared to the manual PathoProof DNA extraction.

Conclusions
The sensitivity and repeatability of PathoProof KingFisher Duo DNA extraction method is statistically comparable to the small manual PathoProof DNA extraction method and to PathoProof KingFisher Flex method. Furthermore, differences in amplification of the internal control suggest the KingFisher Duo method purifies samples from PCR inhibitors more efficiently than the manual PathoProof DNA extraction method or PathoProof KingFisher Flex DNA extraction method. The simplicity of the PathoProof KingFisher Duo DNA extraction protocol increases user-friendliness and the reduced time taken for DNA extraction creates significant time savings and releases resources.
Abstract
The PathoProof™ KingFisher™ Flex DNA extraction kit provides a semi-automated DNA extraction protocol for faster and more convenient sample preparation. The sensitivity and repeatability of the KingFisher Flex method are statistically comparable to the manual PathoProof DNA extraction. The PathoProof KingFisher Flex DNA extraction method purifies samples from PCR inhibitors more efficiently than the manual PathoProof DNA extraction method.

Picture 1. Both total time of extraction and the hands-on time were significantly shorter with KingFisher Flex DNA extraction compared to the manual PathoProof DNA extraction.

Conclusions
The sensitivity and repeatability of PathoProof KingFisher Flex DNA extraction method is statistically comparable to the manual PathoProof DNA extraction method. Furthermore, differences in amplification of the internal control suggest the KingFisher Flex method purifies samples from PCR inhibitors more efficiently than the manual PathoProof DNA extraction method. The simplicity of the PathoProof KingFisher Flex DNA extraction protocol increases user-friendliness and the reduced time taken for DNA extraction creates significant time savings and releases resources.
Development of AMS worldwide, within the Nordic countries and Iceland

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Since the first farms with AMS (Automatic Milking System) started in Holland in 1992, there has been a dramatic change in the development of both the milking technique and the general environment for milk production in the whole world (see graph 1). At the end of 2012 there were AMS on 16,200 farms around the world and it is estimated that the number of AMS single cow units was between 26-27 thousand (Koning, 2013).

Graph 1. Development of AMS worldwide and within the Nordic countries since 1992 (Koning 2013 & NMSM, 2013).

For the Nordic countries it all started with three farms in Denmark in 1997 and now, 15 years later, the total number of farms are more than 3,500 and the milk production from those farms counts for about 25%. Furthermore it is estimated that about 23% of the cows within the Nordic countries are milked with an AMS. Despite the high ratio of both milk and cows only 11.5% of the farms do have AMS in the Nordic countries, meaning that the AMS farms are much bigger on average than other farms. Of course the situation is quite different from country to country but in Iceland the situation is quite unique.
At the end of 2012 the dairy farms in Iceland with AMS was counted for 37,2 million kg. of milk which is 29,7% of the total milk sold to the dairy plants. Probably no other country in the world has this high ratio of the total production. Behind this production stands 125 AMS boxes in 105 farms (1,17 boxes pr. farm) out of 660 dairy farms (15,9%). According to calculations from the cattle data recording system in Iceland there were on average 64,6 dairy cows at each farm with AMS (55,2 pr. AMS box) compared to 34,0 dairy cows at other type of farms at the end of 2012 (52,6% of the AMS farms size). Totally 6.785 dairy cows are stalled in an farm with AMS, counting for 26,4% of all the Icelandic cows (Sigurdsson, 2013).

Each farm with AMS produced in 2012 354 thousand liters of milk when other dairy farms in Iceland produced 158 thousand liters, which is only 44,8% of the production from farms with AMS. The difference is bigger than between the size of the farms (52,6%) which can be explained by higher average yield of the cows milked by an AMS. On average the yield of Icelandic dairy cows was 5.606 kg in 2012, but 6.020 kg for cows in farms with AMS and 5.444 in other type of dairy farms (Sigurdsson, 2013).

On average each AMS box produced (sold to the dairy plant) 302.077 liters of milk (3,1% more than in 2011) but the variation is big, from 117.755 to 444.757 liters. The total production possibility from AMS systems in Iceland in 2012 (from at technical point of view) was 54,7 million liters (43,8% of the country’s total production) (Sigurdsson, 2013).

References:
Penicillin resistance of staphylococci isolated in mastitis - recent development

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²Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Helsinki, Saarentaus, Finland

Materials and methods

Penicillin resistance of 1846 Staphylococcus aureus and 1868 coagulase negative staphylococci (CNS) isolated in clinical and subclinical mastitis during 1988–1989, 1996–1997, 2003–2006 and 2010–2011 were investigated. Milk samples from commercial dairy herds were mainly collected by veterinarians during the routine practice of the Veterinary Teaching Hospital, Food animal unit, University of Helsinki, Finland. Milk samples were examined using conventional methods (Hogan et al., 1999). A minimum of 5 colonies of the same type were defined as positive for intramammary infection, except for S. aureus for which ≥1 colony was used. In 1988–1989 S. aureus were separated from CNS using a slide test (ANI™, Ani Biotech Oy, Finland or Slidex Staph-Plus, BioMerieux, France); since 2006 a tube coagulase rabbit plasma test (BBL™ Coagulase plasma, BD, USA) was used. Susceptibility to benzylpenicillin of the isolates was investigated during 1988–1989 with a disc diffusion test. Since 1996 a nitrocefin test (Nitrocefin, Oxoid, England) was used to determine β-lactamase production of the isolates, which indicates penicillin resistance.

Results and conclusions

Penicillin resistance of S. aureus has reduced during the recent decades, while resistance in CNS seems to have increased. Explanations can be speculated. Use of antibiotics and management of cows with mastitis have changed. Broad-spectrum and combination products have disappeared from treatment and mostly penicillin alone is used. Culling of cows with chronic S. aureus mastitis has become the option of choice. Most of CNS infections are not treated during lactation.

Figure 1. Penicillin resistance of S. aureus (A) and CNS (B) isolated from mastitis during 1988-2011 in the practice area of Veterinary Teaching Hospital in Finland.

References