Diagnostic test properties of a Real-time mastitis test of composite milk samples from milk recordings to identify intramammary infections with Staphylococcus aureus and Streptococcus agalactiae

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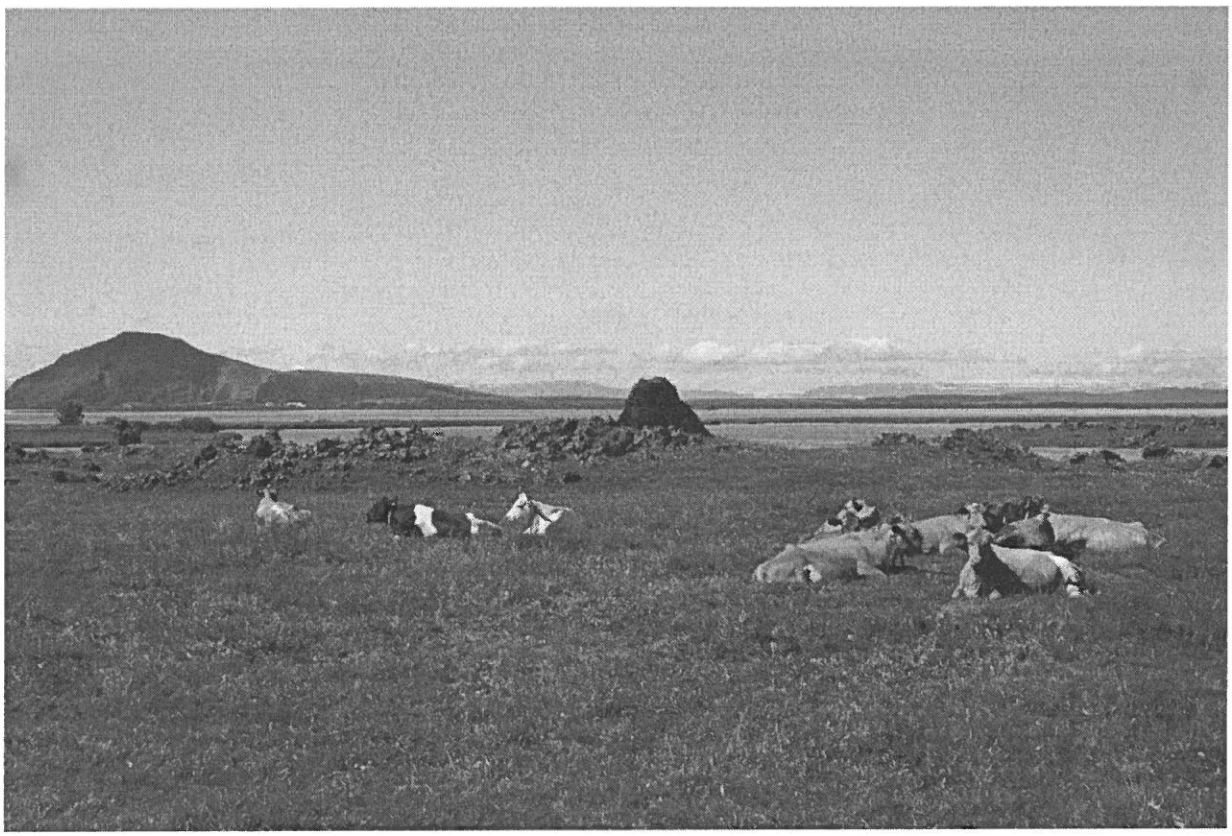

13-14th May 2013, Reykjavik, Iceland

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

The 29th NKVet Symposium: *Mastitis – new knowledge on diagnostics and control on modern dairy farms.* Grand Hotel Reykjavik, Iceland.

Program

Monday 13th May, 2013

9.00-9.15 Opening: Tuula Honkanen-Buzalski, NKVet

Session I. The modern Nordic dairy farm	
9.15-10.00 Key note Olav Østerås, Norway	**The modern Nordic dairy farm.**
10.00-10.15 Mari Hovinen, Finland	Udder health in modern dairy farms – associations with management and grouping of cows.
10.15-10.45 Coffee and posters	
Session II. Milking and mastitis	
10.45-11.30 Key note Esa Manninen, Finland	**Milking and mastitis.**
Session III. Diagnostics	
11.30-12.15 Key note 1 Karin Persson Waller, Sweden	**Microbiological diagnostics of udder infections.**
12.15-13.15 Lunch	
13.15-13.30 Heidi Hiitiö, Finland	Real-time polymerase chain reaction and conventional culture in bacteriological diagnostics of bovine mastitis – a comparative study
13.30-13.45 Ilka C. Klaas, Denmark	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 Ann-Kristin Nyman, Sweden	Diagnostics of intra-mammary bacterial infections – comparison between a PCR assay and culturing.
14.00-14.45 Key note 2 David Eckersall, UK	**Diagnostics for mastitis: opportunities from omics**
14.45-15.00 Ann-Kristin Nyman, Sweden	Associations between cow factors, intra-mammary infections and inflammatory indicators.
15.00-15.30 Coffee and posters	
Session IV :Comparative aspects	
15.30-16.15 Key note Chris Knight, Denmark	**Mastitis in non-bovine dairy species, companion animals and breastfeeding women**
16.15-16.30 Liv Sølverød, Norway	Udder health in Norwegian dairy goat herds.
16.30-16.45 Karin Persson Waller, Sweden	Subclinical mastitis and intra-mammary infections in Swedish beef cows.
19.00 Gala dinner at Restaurant Kolabrautin at the Harpan	

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*

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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.