Danish experiences with a real-time PCR mastitis test of composite milk recording samples to diagnose intramammary infections with Staphylococuus aureus and Streptococcus agalactiae

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Published in:
Proceedings

Publication date:
2014

Document version
Early version, also known as pre-print

DANISH EXPERIENCES WITH A REAL-TIME PCR MASTITIS TEST OF COMPOSITE MILK RECORDING SAMPLES TO DIAGNOSE INTRAMAMMARY INFECTIONS WITH *STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS AGALACTIAE*

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Introduction

Danish farmers can order PCR analysis of the routinely collected milk recording samples using the real-time PathoProof™ Mastitis PCR Assay (Thermo Fisher Scientific Inc., Vantaa, Finland). PCR analysis is mainly used for individual cows pre dry-off or for herd screenings, e.g. in relation to control of contagious mastitis. The test has a high analytical sensitivity (Se) and specificity (Sp) (Koskinen et al. 2009), but knowledge on the performance on composite samples at milk recording is sparse. The aim of our studies was 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 collected samples, carry-over between cows and false positive cows due to teat canal infections, teat skin infections, colonization and/or contamination may occur. Therefore, a further aim was to investigate if pre-sampling procedures and milking order affected the PCR test results.

Material and Methods

A total of 1,199 dairy cows from six herds infected with *S. agalactiae* (study A) and 142 pre dry-off cows from 7 herds (study B) were included. Bronopol preserved composite milk samples were taken at the routine milk recordings between March and May 2011 after the farm personnel had carried out their routine pre-milking practices. All samples were tested using the PathoProof Mastitis PCR assay and results recorded as Ct-values. Sterile quarter foremilk samples were subjected to bacteriological culture (BC) for *S. aureus* in all pre dry-off cows (study B, Cederlöf et al. 2012) and for *S. agalactiae* in 50% randomly selected cows in study A (Mahmmod et al. 2013), i.e. all cows milked at every other milking unit. Data on parity, somatic cell counts (SCC), days in milk (DIM), and kg energy corrected milk (ECM) were obtained from the Danish Cattle Database.

Latent class models (LCA) were used to estimate Se and Sp 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 including herd as random effect was used to investigate the effect of teat disinfection on *S. aureus* PCR positivity in the 6 herds from study A. The effect of milking order on *S. agalactiae* PCR positivity was

investigated in four herds from study A using a logistic regression with generalized estimating equations.

Results and Discussion

Se of the PCR test at Ct-value cut-off 37 were 0.93 for *S. aureus*, and 0.92 for *S. agalactiae* and for both pathogens higher in comparison to Se of BC. For both pathogens, the Se for PCR increased when the Ct-value cut-off increased, whereas the Se for BC decreased with increasing PCR Ct-value cut-off. That changes in the Ct-value cut-off affected the Se of BC could indicate that the underlying disease condition changed from '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 foremilk sample for BC, reduced the odds for positivity in the PCR test significantly for *S. aureus* (odds ratio of 0.75), but not for *S. agalactiae*. These findings may indicate that contamination, teat skin colonization and or teat canal infections increases the risk of false *S. aureus* positive cows in non-sterile taken samples for PCR. A likely explanation is that *S. aureus* may colonize the teat skin, whereas *S. agalactiae* does not. The correlation between consecutively milked cows was 13%, 11%, 9% at *S. agalactiae* Ct-value cut-offs 39, 37, and 34, respectively, suggesting that some carry-over does occur.

Conclusion

Composite milk samples from milk recording can be used for PCR diagnostics of *S. agalactiae* and *S. aureus*, but awareness of differences in Ct-values and risk of carry-over is requiered. The Ct-value cut-off should be chosen according to the purpose of the sampling, i.e. whether identification of all positive cows or identification of heavily/truly infected cows is of interest. Teat disinfection is recommended to reduce false positive results for *S. aureus*. Minor carry-over effects for *S. agalactiae* could be handled by accounting for milking order, repeated tests of positive cows and/or by considering other inflammation markers.

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NMC

A global organization for mastitis control and milk quality

National Mastitis Council 53rd Annual Meeting

Proceedings

January 26 -28, 2014
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