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EFFECT OF PRE-SAMPLING PROCEDURES ON REAL-TIME PCR USED FOR DIAGNOSIS OF INTRAMAMMARY INFECTIONS WITH *STAPHYLOCOCCUS AUREUS* IN DAIRY COWS AT ROUTINE MILK RECORDINGS

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Introduction

Occurrence of teat skin and teat canal bacteria may increase the chance of intramammary infections (IMI) or a false IMI diagnosis even if no IMI occurs. *Staphylococcus aureus* (*S. aureus*) is known to adhere strongly to the mammary epithelial cells (Ditcham et al., 1996). This adherence may increase the risk of false positives, leading to unnecessary culling or overtreatment with antibiotics.

Bacterial culture (BC) has been the primary diagnostic for cow-level diagnosis of *S. aureus* IMI, but this method is less sensitive compared to the faster alternative, PathoProof Mastitis PCR Assay (Thermo Fisher Scientific, Vantaa, Finland), a multiplex real-time PCR technique developed by Koskinen et al. (2009). Danish farmers has since 2010 been able to order cow-level PCR tests based on milk samples collected automatically during the routine milk recordings (Katholm, 2010). Sampling is not performed aseptically, which is in contrast to sampling for BC, where pre-sampling procedures (PSP) including treatment of the teat ends with 70 % alcohol. The objective of this study was to investigate the effect of PSP on the PCR-positivity at cycle threshold (Ct) value ≤ 37 of real-time PCR assay to detect *S. aureus* from composite milk samples at routine milk recordings while accounting for known cow-level risk factors.

Material and Methods

A total of 1,199 dairy cows from six herds with conventional milking parlours were included in this study. Composite milk samples were taken at the routine milk recording between 28th March and 28th May 2011 after the farm personnel had carried out their routine pre-milking practices. The samples were tested using the PathoProof Mastitis PCR assay. Following the farmers' routine pre-milking preparations, 624 cows of the 1,199 cows were randomly selected for BC preceded by PSP. The PSP procedures included cleaning of teats, removing the first streaks of milk and 70 % alcohol teat disinfection. Data on parity, somatic cell counts (SCC), days in milk (DIM), and kg energy corrected milk (ECM) were also obtained. 'PCR-positivity for *S. aureus*' was used as outcome variable with the PSP being the primary explanatory variable and SCC, DIM and ECM and parity secondary variables. Statistical analyses were carried out using a generalized hierarchical logistic mixed model, where herd was included as a random effect to account for within-herd clustering.

Results and Discussion

The within herd test prevalence was 31 %, ranging from 16 % to 48 % for the six herds. Univariable analysis showed that the PSP, SCC, ECM, and DIM were significantly associated with PCR-positivity, while parity was not. The resulting final model consisted of the variables PSP and SCC, with a random herd effect accounting for 8.9% of the total variation.

Pre-sampling procedures

Teat skin, teat canal, and teat orifices are the key points for bacterial introduction and invasion. Therefore, PSP can reduce the quantity of *S. aureus* that contaminates and colonizes these areas (Bramley et al., 1979). Regardless of the farmers' pre-milking practices, the PSP carried out for BC decreased the chance of being PCR-positive to 0.75 (95% CI; 0.58 - 0.97) compared to cows where the PSP were not carried out. This significant association could mainly be the result of alcohol teat disinfection, the physical effect of discarding the first two to three streams of milk before sample collection and the five ml milk sample taken for BC from each quarter.

Pre-milking practices

The farm personnel's pre-milking practices may have reduced the amount of teat skin bacterial cells of *S. aureus* in both groups of cows. None of the six herds used any pre-milking teat dips or chemicals with teat disinfecting abilities that could have reduced the amount of *S. aureus* in general. However, routine pre-milking practices and post-milking dipping could be important procedures for reduction of the quantity of bacterial cells, but they are not sufficient to avoid contamination of milk samples collected for PCR testing. Therefore, it would be preferable to perform PSP before sampling for PCR testing for *S. aureus* IMI detection.

Conclusion

We demonstrated that PSP in connection with BC decrease the cow's chance of having a low PCR Ct value for *S. aureus* IMI. PSP may improve the specificity of PCR tests used on milk samples collected at routine milk recordings and should be performed before PCR sampling.

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