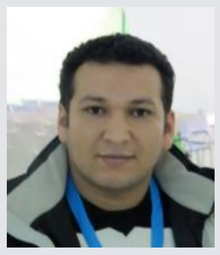
Bayesian estimation of test characteristics of Real-time PCR, bacterial culture and California Mastitis Test for diagnosis of *Staphylococcus aureus* in dairy cattle



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Background & Objective

- Danish farmers can order the Real-time ***PathoProof***™ Mastitis PCR Assay on non-sterile taken composite samples from routine milk recordings.
- PCR has high analytical sensitivity and specificity, but is not evaluated on composite samples from milk recording.
- Bacterial culture (BC) is commonly used for identifying *Staphylococcus aureus* (SA) however, it may be associated with some limitations.
- California Mastitis Test (CMT) is a quick, cheap, and simple cow-side test for identification of subclinical mastitis under field conditions.
- ***The objective:*** To estimate the sensitivity (Se) and specificity (Sp) of real-time PCR, BC and CMT for the diagnosis of the naturally occurring intramammary infections (IMI) with SA in routinely collected milk samples using latent class analysis (LCA) to avoid the assumption of a perfect reference test.

Methodology

- 609 dairy cows randomly selected from 6 herds with milking parlours and with bulk tank PCR Ct value ≤ 39 for SA.
- At milk recordings, quarter foremilk samples aseptically collected for BC and CMT, while composite samples was collected for PCR, figure (1).
- Cow considered BC-positive, when 2 colonies of SA were identified in at least one quarter.
- Cow considered CMT-positive, when at least one quarter has a score ≥ 3.
- Cow considered PCR-positive, when Ct-value was ≤ 37.
- LCA models with different scenarios for conditional dependence (COC) and independence (CID) were run for the 3 tests.
- LCA was performed by introduction the model into OpenBUGS software.

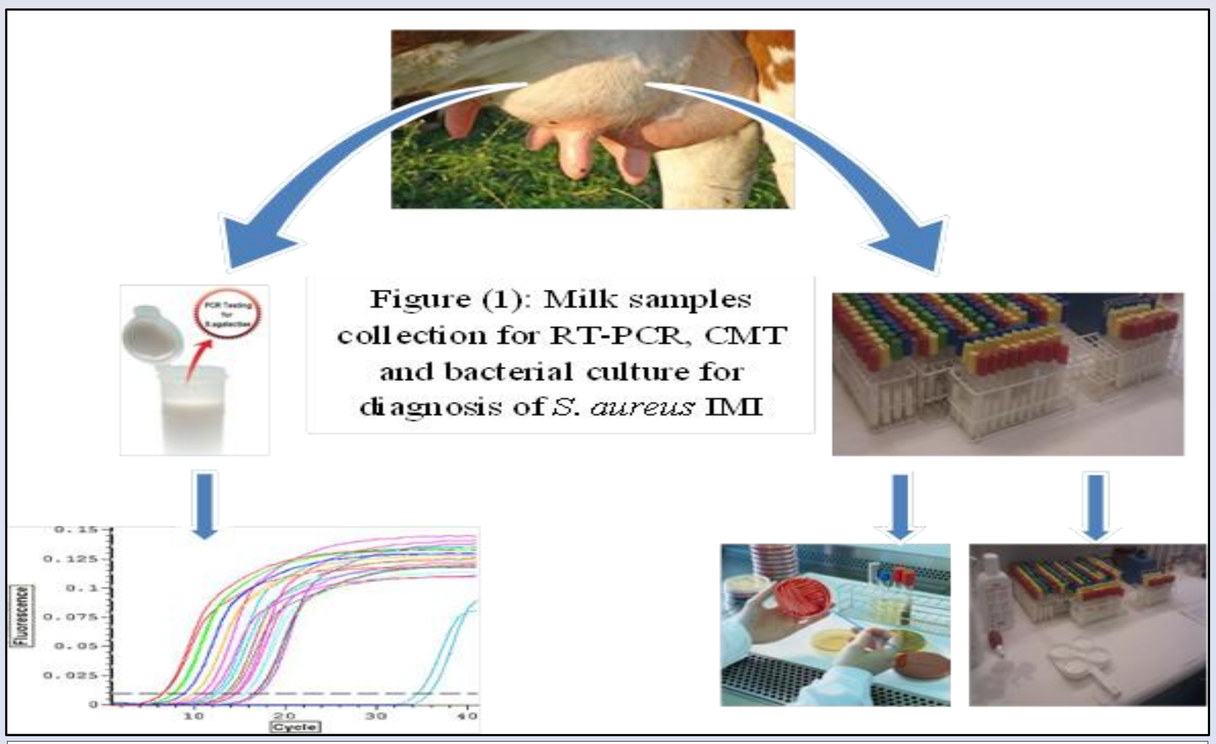

Figure (1): Milk samples collection for RT-PCR, CMT and bacterial culture for diagnosis of *S. aureus* IMI

Results

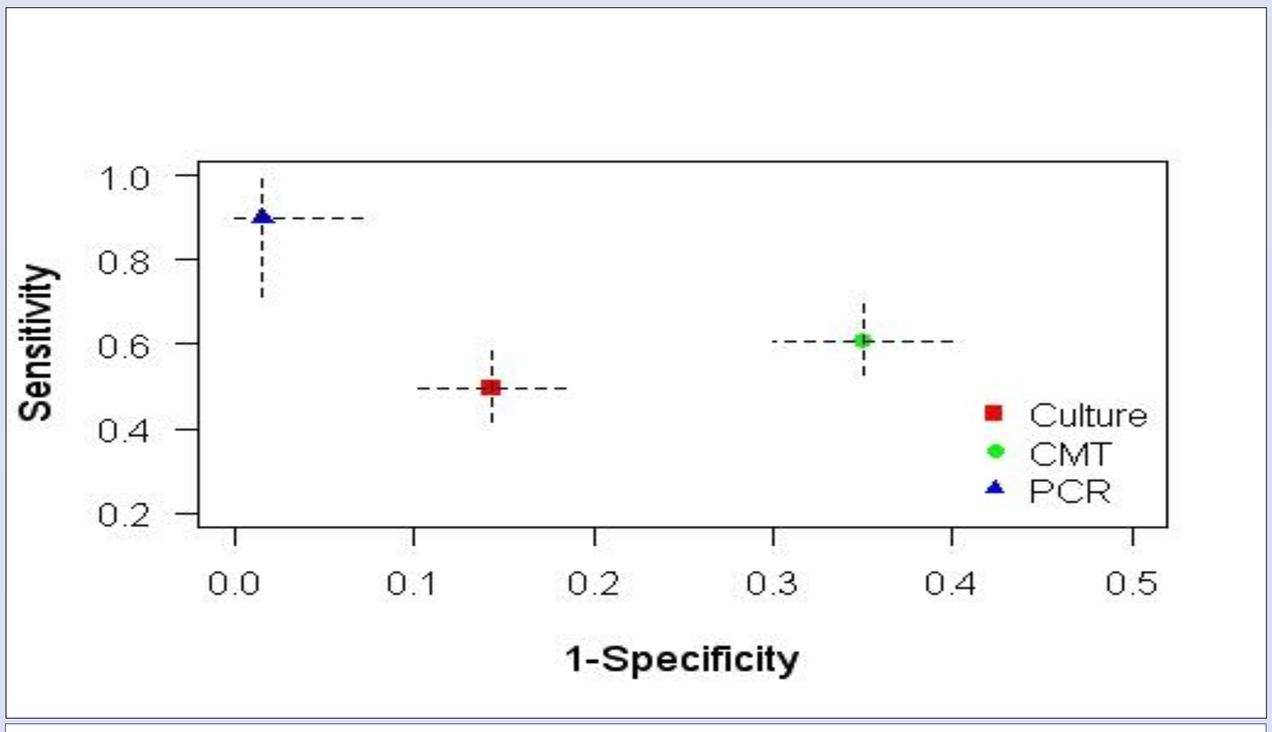

Figure 2: Test estimates (Se, Sp) for PCR, CMT and BC at PCR cut-off ≤ 37 for diagnosis of SA IMI in 6 herds estimated using LCA.

Figure 1: Collection of milk samples from 6 dairy herds for diagnosis of SA IMI by PCR, BC and CMT at milk recording.

- The prevalence of SA IMI based on BC was 25% and 28% based on PCR.
- SePCR was 89%, while SeBC was 50%, and SeCMT was 61%.
- SpPCR was 98%, while SpBC was 86%, and SpCMT was 65%.
- Estimates of PCR were higher than the estimates of BC and CMT, figure (2).
- SeCMT was higher than SeBC however, SpBC was higher than SpCMT.
- Based on the DIC, the model assuming COC between BC and CMT was preferred (DIC= 266.3), over other models scenarios.

Discussion

Differences between PCR, CMT & BC

- Differences in inoculum size
- Differences in sample type
  - Quarter vs. composite
  - Foremilk vs. whole milk
- Different biological principles for PCR, CMT& BC
- Effect of observer
- Low Se and Sp of CMT due to unspecific reaction to inflammatory cells and bacterial cells other than SA.

Factors influencing PCR estimates

- Highest Se and Sp for PCR because detection of viable and non-viable SA
- Risk of false positives possible because of
  - SA colonizing teat skin and canal?
  - Carry-over between subsequently milked cows?

Factors influencing BC estimates

- Intermittent shedding of viable SA
- Definition of SA IMI (2 colonies) on BC
- Contamination during sampling or processing in the lab (47 quarter samples classified as contaminated)

Conclusions

✓ PCR has a higher accuracy than CMT and BC suggesting its usefulness as diagnostic test of IMI with SA from dairy cows at routine milk recordings.

✓ Further research may be necessary to evaluate the risk of contamination and carry-over.

SVEPM 2013 Madrid, Spain