UABDIVULGA
BARCELONA RECERCA I INNOVACIÓ

27/02/2019

New detection method of Contagious Mastitis Pathogens in cows

Contagious mastitis is an infection of the mammary glands (udders) of cows and it is caused by the *Streptococcus agalactiae* and *Staphylococcus aureus* pathogens. This disease is a major problem in our society from an economic and public-health point of view. That is why, accurate diagnostic screening tests for early detection of these pathogens are essential in order to take measures to reduce the risk of new infections. So far, bacterial culture has been the standard method to identify these pathogens. However, this study proves that qPCR is a valuable method to detect both pathogens due to its sensitivity and specificity concluding that it should be considered in the current routine diagnostic tests.

Intramammary subclinical infections (IMI) with *Streptococcus agalactiae* (*Strep. agalactiae*) and *Staphylococcus aureus* (*Staph. aureus*) are a major problem in dairy herds from an economic, diagnostic and public-health related point of view. The contagious udder pathogen *Staph. aureus* is widespread in dairy herds, and despite successful control efforts to reduce *Strep. agalactiae* in Scandinavian countries during the 20th century; the proportion of positive herds increased throughout the early 21st century. The control of bovine mastitis caused by *Staph. aureus* and *Strep. agalactiae* mainly depends on prevention of new infections within and between the dairy

herds through maintenance of proper biosecurity measures. The transmission of these contagious mastitis pathogens occurs primarily during milking. Milking hygiene and teat cleaning in automatic milking system (AMS) differ from the conventional milking system, with more cows per milking unit and no contact with human hands. Therefore, a difference in the prevalence of *S. aureus* and *S. agalactiae*, isolated from both milk and teat skin in AMS compared to conventional milking systems, is expected.

Even though both bacteria are known as contagious mastitis pathogens, environmental reservoirs are reported in the scientific literature. A faeco-oral transmission cycle may perpetuate and amplify the presence of *Strep. agalactiae* within the dairy herds but the importance of these environmental reservoirs is still being discussed. This, together with the environmental reservoir of contagious mastitis pathogens, could explain why *Staph. aureus* remains a problem, and why there has been a re-emergence of *Strep. agalactiae* in line with an increase in the proportion of farms using AMS in Denmark.

Monitoring udder health performance is impossible without reliable and affordable diagnostic methods. Therefore, accurate diagnostic screening tests for early detection of pathogen specific subclinical mastitis are essential to initiate the appropriate treatment or culling to separate infected animals and to take measures to reduce the risk of new infections within herd or prevent introduction in new herds. So far, bacterial culture (BC) has been the reference standard for identification of mastitis pathogens but due to the higher sensitivity and quick results, PCR is gaining more footage in the udder health monitoring. Furthermore, use of molecular methods as part of mastitis diagnostics could contribute to targeting of transmission prevention measures.

The bovine teat skin may be an important reservoir for contagious mastitis pathogens, as the presence of bacteria on teat skin has been associated with IMI in the same quarter and bacteria has been found on teat skin in quarters not having IMI, suggesting that colonization and/or contamination of teat skin from sources other than milk of the same quarter is likely possible. Furthermore, controlling of *Staph. aureus* and *Strep. agalactiae* in large dairy herds without considering the environmental reservoirs may lead to unsuccessful control and eradication. Therefore, PCR tests on teat skin or environmental samples could become a useful tool in controlling *Strep. agalactiae* and *Staph. aureus* mastitis.

We carried out this study to estimate the sensitivity (Se) and specificity (Sp) of the commercially available Mastit4 qPCR assay and BC for the identification of *Strep. agalactiae* and *Staph. aureus* in milk and teat skin samples from high somatic cell count (SCC) cows in AMS herds using latent class model (LCA) fit within a Bayesian framework.We randomly selected 30–40 cows and teat skin samples and aseptic milk samples were collected. Bacterial colonies of *Strep. agalactiae* and *Staph. aureus* were isolated on different bacterial culture agars and then were phenotypically identified based on morphological characteristic.

Our findings from the milk samples analysis showed that the Se and Sp of qPCR for *Strep. agalactiae* were estimated to 97% and 99%, respectively, whereas the Se and Sp of BC were 41% and 100%, respectively. The Se and Sp of qPCR for *Staph. aureus* were estimated to 95% and 99%, respectively, whereas the Se and Sp of BC were 54% and 77%, respectively. For teat skin samples, the Se and Sp of qPCR for *Strep. agalactiae* were estimated to be 97% and 96%, respectively, whereas the Se and Sp of BC were 33% and 100%, respectively. The Se and Sp of qPCR for *Staph. aureus* were estimated to 94% and 98%, respectively, whereas the Se and Sp

of BC were 44% and 74%, respectively.

We concluded that the Se for diagnosing *Strep. agalactiae* and *Staph. aureus* IMI was higher for qPCR than BC, suggesting that qPCR is a valuable method for detecting both pathogens from quarter-level milk samples. The performance of BC in the detection of *Strep. agalactiae* and *Staph. aureus* on teat skin was poor compared to qPCR, indicating that differences in the target condition of the two methods should be considered when implementing them as routine diagnostic tests for detecting teat skin colonisers. The low Se of BC may preclude the use of BC for skin testing, and qPCR is better for this task.

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References

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