Association between teat skin colonization and intramammary infections with *Staphylococcus aureus* and *Streptococcus agalactiae*

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Introduction and Objective

Staphylococcus aureus and *Streptococcus agalactiae* are traditionally considered contagious mastitis pathogens mainly causing subclinical infections in dairy cattle. The consequences of subclinical mastitis are economic losses to the farmer and impaired milk quality. To improve the control of contagious mastitis pathogens, knowledge of reservoirs and transmission is crucial. The role of the teat skin as a reservoir of *Strep. agalactiae* is unknown and still discussed for *Staph. aureus*. Furthermore, teat skin colonization is not yet investigated in automatic milking systems (AMS) with more milkings per cow and less human contact compared with conventional milking. Thus, the objective of this study was to investigate the association of teat skin colonization with intramammary infections for *Staph. aureus* or *Strep. agalactiae* in herds with AMS.

Materials and Methods

Lactating cows (n=300) with somatic cell count ≥ 200.000 cells/mL at last milk recording before sampling were selected from eight AMS herds with *Strep. agalactiae* positive bulk tank milk. Quarter foremilk samples were collected aseptically according to guidelines of the National Mastitis Council. Teat skin samples were collected using the "wet-dry method" where a wet and a dry rayon swab is rotated 360° around the teat approximately 1 cm from the teat canal orifice and both swabs are immersed into the same tube containing 2 mL ¼ Ringer solution. Samples were kept at 5°C until bacteriological culture was performed the following day. All samples were cultured on blood agar, Modified Edward's medium and a selective medium for *Staph. aureus*. After homogenization, 0.1 mL of a teat skin sample was inoculated to a whole agar plate and 0.01 mL of milk was streaked on a quarter of an agar plate. Plates were incubated aerobically at 37°C for 48 h. Identification was based on morphology and all suspected colony-types were verified using MALDI-TOF or latex agglutination test. Positive samples were defined as: at least one medium with ≥ 1 colony of *Staph. aureus* or *Strep. agalactiae*.

Results and Conclusion

Out of 1142 quarter milk samples from 300 cows with increased somatic cell count the prevalence of *Staph. aureus* and *Strep. agalactiae* was 8.1 % and 7.4 %, respectively. In teat skin samples the prevalence of *Staph. aureus* and *Strep. agalactiae* was 6.6 % and 0.35 %, respectively. *Strep. agalactiae* was isolated from teat skin, however, only in four quarters from two cows also having *Strep. agalactiae* in milk. For *Staph. aureus*, we calculated a 3.2 higher odds of a quarter being milk positive and teat skin positive, relative to being teat skin negative and milk positive ($P < 0.05$). This relationship between *Staph. aureus* on teat skin and in milk was also supported by our preliminary multivariate (log-linear) analysis.

In conclusion, *Strep. agalactiae* can be isolated from the teat skin of cows with *Strep. agalactiae* in milk, but it appears to be very uncommon even in high-risk herds and cows. For *Staph. aureus* there is considerable evidence of interrelation between teat skin colonization and intramammary infection.