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FOOD SAFETY

Fight Mastitis

Staphylococcus aureus

Genotype B and its detection

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Analytics so far *Staph. aureus*

Staphylococcus aureus (***Staph. aureus***) is the most common cause of contagious mastitis in cattle worldwide (Schällibaum, 1999; Zecconi et al., 2005). In Switzerland, it causes great economic loss (Kirchhofer et al., 2007), and control of this pathogen would be very beneficial. Unfortunately, conventional routine bacteriology as a diagnostic tool is not entirely satisfactory, as the overall diagnostic sensitivity in single milk samples reaches only 79.9% (21.4 to 100%) (Studer et al., 2008), because this germ is cyclically shed in milk from the cow's udder. As a consequence, triple consecutive sampling is necessary to achieve a satisfactory diagnostic sensitivity (Sears et al., 1990), but it is often too expensive and time consuming, so that normally routine testing is accomplished with single analysis. For this, quarter or four-quarter milk samples were used so far. The use of bulk tank milk (**BTM**) was not suitable due to too low specificity, as *Staph. aureus* in BTM may also originate from other sites than the mammary gland (environmental *Staph. aureus*) including teat and udder skin, parlor, milking machine, air and bedding (Sears and McCarthy, 2003) and contaminations lead to false results and conclusions. And furthermore, there is only a weak association between colony forming units of *Staph. aureus* measured in BTM and the number of infected cows (Farnsworth, 1993; Gonzalez et al., 1986). From a clinical point of view, this approach is not acceptable as many cows falsely remain undetected and permit other cows to be infected.

Developments

Based on this facts, Graber et al. (2007) developed a highly sensitive and specific assay to extract and detect *Staph. aureus* in raw milk samples, based on molecular biology. It detects the *nuc* gene, typical of *Staph. aureus*. Its potential for automation and routine examinations is the basis for a wide range of use. This assay is more than 500 times more sensitive than conventional bacteriology and is highly specific for *Staph. aureus* (100%). It was evaluated in a longitudinal field study by Studer et al. (2008) and showed a diagnostic sensitivity of 99.4% and a diagnostic specificity of 97.1%.

Genotypes

Lateron, in the studies of Fournier et al. (2008) and Graber et al. (2009), various genotypes of *Staph. aureus* with different virulence and pathogenicity factors were identified and described. Genotype B (**GTB**) and genotype C were predominant in Swiss dairy herds, whereas the remaining genotypes were rarely found. *Staph. aureus* GTB was related to high contagiosity and increased pathogenicity, causing herd problems with cow prevalences up to 87% (Graber et al., 2009). In contrast, genotype C and others than were found with infections of single cows,

and mostly affected only one single quarter of the udder. Genotyping by Fournier et al. (2008) showed a high association between genotypes and virulence gene patterns. Among others, *Staph. aureus* GTB was characterized by the presence of the *Staph. aureus* enterotoxin genes A (***sea***) and D (***sed***), and by a polymorphism within the leucotoxin E gene (**lukEB**), caused by a point mutation.

As shown by Fournier et al. (2008) and Graber et al., 2009), *Staph. aureus* GTB infects many cows in a herd and requires infected herds to be sanitized in order to reduce SCC at herd level and enhance milk quality. From clinical experience we know that proper sanitation alone takes 1 year and is expensive because of treatment costs, loss of milk, culling, replacements, additional work for the farmers and veterinary support. For minimizing intramammary infection caused by *Staph. aureus* GTB at the herd level, but also at the country level, detection and monitoring of *Staph. aureus* GTB-positive herds would be most efficient and most economic by analyzing BTM. This sample type is convenient, as the sample technique is fast and easy, compared to the sampling of quarter milk samples under aseptic conditions, favorably done by a veterinarian.

To further improve the diagnostics for *Staph. aureus* as a mastitis pathogen, it was the goal of the study of Boss et al. (2011) to develop a novel assay for to detect the contagious genotype B of *Staph. aureus* in BTM. It includes preparation of bacteria from milk as described by Graber et al. (2007) and a real-time quantitative Polymerase Chain Reaction (**qPCR**) for the three GTB-typical targets sequences lukEB, *sea* and *sed* (Fournier et al., 2008a; Graber et al., 2009). A BTM sample is defined as GTB-positive, if qPCR for lukEB and *sed* and/or *sea* generates a positive result each ≥ 10 copies/reaction. Quantification is based on the lukEB target. The method showed a high analytical sensitivity and specificity. Based on simulation experiments, one infected among 138 healthy cows can theoretically be detected. Furthermore, the method showed a high repeatability, is characterized by a small intra- and interassay variability and permits detection of targets within a wide dynamic range, rendering dilution steps of samples unnecessary.

BTM was used as it is the most efficient and economical way of detecting and monitoring GTB-positive herds. Once a herd is detected, sanitation requires all the cows to be tested by the same assay to separate healthy from infected cow. This is by far the most important step for successful sanitation (Kirchhofer et al., 2011).

Once a herd is sanitized, the BTM analysis can be used to monitor the herd in the following, so that new GTB infections can be detected in an early phase, when only one or few cows are affected. Furthermore, the cure rate in newly infected cows is higher (Gruet et al., 2001) and use of antibiotics is reduced compared to cows with a long time infection. SCC analysis alone is less vialbe for *Staph. aureus* GTB screenings as there are many other causes for increased SCC.

An extensive field study was performed for evaluating the newly developed analytical method, extended with an enrichment step (Syring et al., 2012) by comparing to an reference method. Therefore 21 *Staph. aureus* GTB-positive and 33 *Staph. aureus* GTB-negative herds were evaluated. Different sample types were evaluated in order to find the differences: (1) samples, taken under aseptic conditions by a veterinarian; (2) BTM; and (3) samples from the official quality control system, commonly taken once a month in Switzerland by instructed people. The results showed, that the new method gives the same results with high sensitivity and specificity, and that, furthermore, all sample types could be used. With BTM, there was the only restriction that the results are valid only for cows milked into the tank and not excluded for any reasons. SCC evaluated simultaneously showed similar results as described in the study of Fournier et al. (2008).

Conclusions

The described qPCR analytics is a fast and potent method for screening easily available BTM samples for the *Staph. aureus* GTB. It is characterized by a high analytical specificity and sensitivity as well as by »

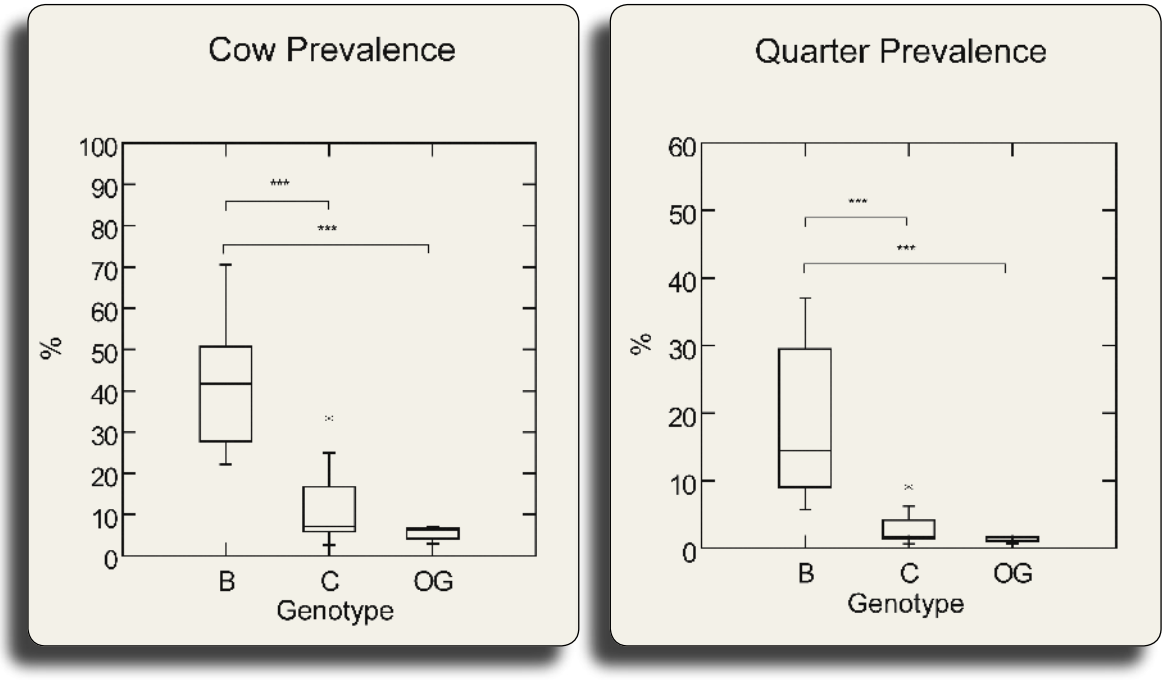

Figure 1. Cow and quarter prevalences in Swiss dairy herds depending on the genotype of *Staph. aureus* (Graber et al., 2009)

Notes. B: *Staph. aureus* genotype B; C: *Staph. aureus* genotype C; OG: other genotypes than genotype B

a high repeatability. Based on our studies, the methodology provides a powerful tool for the control of this contagious pathogen.

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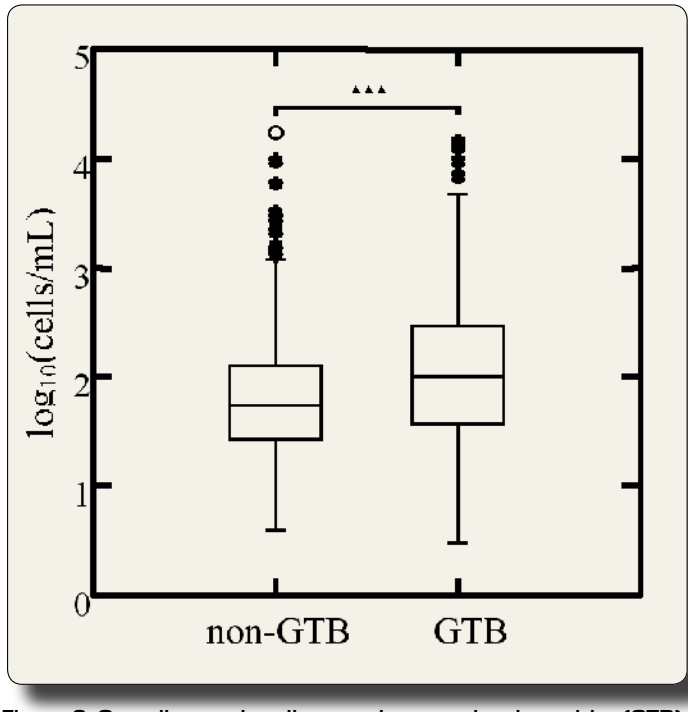

Figure 2. Overall somatic cell counts between herds positive (GTB) and negative (non-GTB) for *Staph. aureus* genotype B, respectively. * outlier; ○ far outlier; ♦♦♦ P < 0.001.