

Bovine Mastitis: selective detection of the contagious genotype B of *Staphylococcus aureus* in bulk tank milk and in samples of breeding programs

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Key words

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Aim of the study

Staphylococcus aureus genotype B (*S. aureus* GTB) is a bacterial pathogen causing contagious, chronic mastitis in cattle and is, therefore, responsible for high costs in the Swiss dairy industry. In the past, an assay was developed (Boss et al. 2010) to detect *S. aureus* GTB in bulk tank milk (BTM). One aim of the study was to evaluate the assay in a field study and to implement the method in a commercial laboratory (Suisselab).

Besides the financial aspects as a contagious udder pathogen, *S. aureus* GTB may also play an important role in the context of food safety of raw milk products as it is frequently present in raw milk cheese which may serve as a source of contamination and infection in humans. The second aim of the study was, therefore, to compare the genome of bovine strains of *S. aureus* to that of human strains and to evaluate in-silico the potential of bovine strains to infect humans.

Material and methods

- Field study: 21 herds positive for *S. aureus* GTB, 33 control herds; qPCR assay for *S. aureus* GTB
- Herd prevalence of *S. aureus* GTB: 223 herd milk samples obtained from the official quality control program; qPCR assay for *S. aureus* GTB
- Implementation of the qPCR assay for *S. aureus* GTB in a commercial laboratory
- Genome evaluation: whole-genome sequencing of 7 bovine strains and comparison to 17 genomes of human *S. aureus* strains present in publically accessible databases

Results and significance

The results and the significance of the project are described in more detail in the “Abschlussbericht zum Forschungsprojekt *Staphylococcus aureus* Genotyp B” by Graber and Boss. In January 2013 the report was distributed to the project sponsors including Agroscope, Bundesamt für Gesundheit, Bundesamt für Veterinärwesen, Fromarte, Schweizer Milchproduzenten SMP, Suisselab, and TSM Treuhand.

- Field study: the novel qPCR assay using BTM turned out to be excellent for detecting GTB positive herds, as all the positive ones were unambiguously discriminated from the negative ones. The level of detection is very low (10 cfu/ml milk) allowing the discovery of at least 1 positive cow among 138 cows in BTM. The assay is automatable and is highly effective at reasonable cost.
- Herd prevalence: a representative stratified sample (n=223 herd milk samples) was analyzed. In total, 10.3% of the Swiss dairy herds were infected by *S. aureus* GTB (end of 2012). As on average 49% of the cows of a herd are infected by this pathogen (Fournier et al., 2008) and the treatment success is low (30%; Gruet et al., 2001), most of the infected cows need to be eradicated. As a consequence, the disease causes high costs in dairy farming nationally (approximately CHF 100 mio./year in Switzerland). An official control program would therefore be of great benefit. The required diagnostic test, the novel GTB qPCR assay, is available and is successfully validated.
- Implementation of the qPCR assay for *S. aureus* GTB in a commercial laboratory: according to the contract with the sponsors it was planned to implement the qPCR assay in a commercial laboratory (Suisselab) and to further optimize the assay to reduce the diagnostic costs. The latter goal was assessed by reducing the number of manual steps and by using a simplified method to extract the bacterial DNA. Implementation in a commercial laboratory needs yet to be done.
- Genome evaluation: *S. aureus* GTB is a contagious mastitis pathogen but own studies recently demonstrated that this agent is also frequently present in raw milk cheese and is able to produce various enter-

otoxins. Furthermore, *S. aureus* GTB is a member of the CC8 cluster which includes a series of strains seriously pathogenic for humans. Whole-genome sequencing of 7 own bovine strains together with bio-informatics and phylogenetic evaluation clearly demonstrated that all the bovine strains actually descended from human-pathogenic strains. In addition it turned out that the 3 GTB strains were highly similar at the sequence level to the well-known USA300 FPR3757 and Newman strains. Both of them are pathogenic for humans and are representatives of CC8. Based on the large number of virulence factors identical between the bovine GTB and the human strains it is assumed that the bovine strains largely retained their pathogenic properties for humans. As a consequence, it is reasonable for the present to consider *S. aureus* GTB as a zoonotic pathogen. To gain a deeper insight into this topic, 2 research projects were started in spring 2013. They are financed by a grant of the Swiss National Foundation dedicated to Prof. P. Moreillon, University of Lausanne, and ALP (H. Graber).

Publications, posters and presentations

See "Abschlussbericht zum Forschungsprojekt *Staphylococcus aureus* Genotyp B" by Graber and Boss

Project 1.11.09, 1.12.10

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