J. Dairy Sci. 97:1–11
http://dx.doi.org/10.3168/jds.2013-7760

Genotype-specific risk factors for *Staphylococcus aureus* in Swiss dairy herds with an elevated yield-corrected herd somatic cell count

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ABSTRACT

Bovine mastitis is a frequent problem in Swiss dairy herds. One of the main pathogens causing significant economic loss is *Staphylococcus aureus*. Various *Staph. aureus* genotypes with different biological properties have been described. Genotype B (GTB) of *Staph. aureus* was identified as the most contagious and one of the most prevalent strains in Switzerland. The aim of this study was to identify risk factors associated with the herd-level presence of *Staph. aureus* GTB and *Staph. aureus* non-GTB in Swiss dairy herds with an elevated yield-corrected herd somatic cell count (YCHSCC). One hundred dairy herds with a mean YCHSCC between 200,000 and 300,000 cells/mL in 2010 were recruited and each farm was visited once during milking. A standardized protocol investigating demography, mastitis management, cow husbandry, milking system, and milking routine was completed during the visit. A bulk tank milk (BTM) sample was analyzed by real-time PCR for the presence of *Staph. aureus* GTB to classify the herds into 2 groups: *Staph. aureus* GTB-positive and *Staph. aureus* GTB-negative. Moreover, quarter milk samples were aseptically collected for bacteriological culture from cows with a somatic cell count ≥150,000 cells/mL on the last test-day before the visit. The culture results allowed us to allocate the *Staph. aureus* GTB-negative farms to *Staph. aureus* non-GTB and *Staph. aureus*-free groups. Multivariable multinomial logistic regression models were built to identify risk factors associated with the herd-level presence of *Staph. aureus* GTB and *Staph. aureus* non-GTB. The prevalence of *Staph. aureus* GTB herds was 16% (n = 16), whereas that of *Staph. aureus* non-GTB herds was 38% (n = 38). Herds that sent lactating cows to seasonal communal pastures had significantly higher odds of being infected with *Staph. aureus* GTB (odds ratio: 10.2, 95% CI: 1.9–56.6), compared with herds without communal pasturing. Herds that purchased heifers had significantly higher odds of being infected with *Staph. aureus* GTB (rather than *Staph. aureus* non-GTB) compared with herds without purchase of heifers. Furthermore, herds that did not use udder ointment as supportive therapy for acute mastitis had significantly higher odds of being infected with *Staph. aureus* GTB (odds ratio: 8.5, 95% CI: 1.6–58.4) or *Staph. aureus* non-GTB (odds ratio: 6.1, 95% CI: 1.3–27.8) than herds that used udder ointment occasionally or regularly. Herds in which the milker performed unrelated activities during milking had significantly higher odds of being infected with *Staph. aureus* GTB (rather than *Staph. aureus* non-GTB) compared with herds in which the milker did not perform unrelated activities at milking. Awareness of 4 potential risk factors identified in this study guides implementation of intervention strategies to improve udder health in both *Staph. aureus* GTB and *Staph. aureus* non-GTB herds.

Key words: bulk milk, *Staphylococcus aureus* genotype B (GTB), risk factor, Switzerland

INTRODUCTION

Staphylococcus aureus is one of the most important contagious mastitis pathogens in dairy cattle and is associated with large economic losses (Halasa et al., 2007; Hogeveen et al., 2011). The bovine mammary gland represents the most important reservoir of mastitis-associated *Staph. aureus* (Sears and Carthy, 2003). Additionally, *Staph. aureus* has been isolated from extramammary sites such as the teat skin, teat orifice, hock skin, housing infrastructure, feedstuffs, skin of milking personnel, insects, nonbovine animals, milking equipment, farm equipment, and bedding material (Fox et al., 2001; Oliver et al., 2005; Piccinini et al., 2009; Anderson et al., 2012).

With the availability of novel molecular methods, several genotypes of S*taph. aureus* have been identified with different epidemiological and biological properties

Received November 25, 2013.
Accepted April 7, 2014.
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(such as different virulence and pathogenicity factors for the different strains; Green and Bradley, 2004; Barkema et al., 2006; Graber et al., 2009). In Switzerland, Fournier et al. (2008) identified 17 strains of *Staph. aureus* by ribosomal spacer (**RS**)-PCR, of which genotypes B (**GTB**) and C (**GTC**) were most frequently diagnosed. Further studies showed that *Staph. aureus* GTB is udder-associated, contagious, and often responsible for herd health problems, as apparent by a high within-herd *Staph. aureus* prevalence (ranging from 18.2 to 87.5%; Graber et al., 2009), whereas other *Staph. aureus* genotypes were associated with a low within-herd *Staph. aureus* prevalence (ranging from 4.0 to 33.3%; Graber et al., 2009) and rarely caused herd health problems (Fournier et al., 2008; Graber et al., 2009; Michel et al., 2011). Furthermore, Fournier et al. (2008) and Graber et al. (2009) found that *Staph. aureus* GTB had specific virulence and pathogenicity factors that were different from those of other *Staph. aureus* genotypes. *Staphylococcus aureus* GTB is characterized by the presence of the enterotoxin genes *sea*, *sed*, and *sej*, a long x-region of the *spa* gene, and a GTB-typical SNP within the *lukE* gene (Fournier et al., 2008; Graber et al., 2009). In contrast, *Staph. aureus* GTC was positive for *sec*, *seg*, *sei*, and *tst*, whereas all the remaining genotypes were heterogeneous in their virulence gene pattern. The described virulence gene patterns highly correlated with the genotypes obtained by RS-PCR (Fournier et al., 2008) and were then used to develop a novel analytical approach based on real-time quantitative PCR (**qPCR**) to detect *Staph. aureus* GTB highly specifically (Boss et al., 2011; Syring et al., 2012).

Although culturing a single bulk tank milk (**BTM**) sample has a low sensitivity for detection of *Staph. aureus* (Francoz et al., 2012), bulk tank milk analysis by PCR is a useful alternative tool for monitoring the udder health status of a herd. It is less expensive, allows for more convenient sampling, and requires less time for laboratory analysis compared with bacteriological culture of quarter milk samples (Jayarao and Wolfgang, 2003; Syring et al., 2012). However, in contrast to aseptically collected quarter milk samples, it is only assumed to be a reliable tool for the monitoring of udder-associated pathogens, because BTM is often contaminated with environmental bacteria (Olde Riekerink et al., 2010). Therefore, Boss et al. (2011) developed and evaluated a qPCR assay for the detection of *Staph. aureus* GTB in BTM as this is assumed to be a contagious pathogen given the high within-herd prevalence reported (Graber et al., 2009).

For effective prevention of IMI, it is important to know the prevalence and distribution of its causative pathogens as well as the pathogen-specific risk factors associated with the disease (Olde Riekerink et al., 2010). Cow-level risk factors for *Staph. aureus* IMI include overmilking, poor teat-end condition, epidermal wounds, a higher parity, infected rear quarters, and an additional quarter infected with *Staph. aureus* within the same cow or herd (Romain et al., 2000; Zadoks et al., 2001; Dufour et al., 2012). Not wearing milking gloves, not following any plausible milking order, no fly control, and no dry cow treatment were identified as important herd-level risk factors for IMI caused by *Staph. aureus* (Erskine et al., 1987; Hutton et al., 1990; Bartlett and Miller, 1993; Moret-Stalder et al., 2009; Dufour et al., 2012). As risk factors differ among mastitis-causing pathogens, they may also differ between different *Staph. aureus* genotypes displaying different epidemiological properties. However, not much is known about genotype-specific risk factors for *Staph. aureus* mastitis.

The aim of this study was to identify risk factors associated with the presence of *Staph. aureus* GTB and *Staph. aureus* non-GTB in dairy herds with an elevated yield-corrected herd SCC (**YCHSCC**).

MATERIALS AND METHODS

Herd Selection

Yield-corrected herd SCC is defined as the calculated arithmetic average herd SCC of all lactating animals in the herd taking into account their individual milk production (Lievaart et al., 2007). This is more accurate and better reflects the subclinical mastitis situation in a dairy herd than samples taken from the BTM, because the milk of some cows is withheld (e.g., withdrawal after antimicrobial treatment) from the bulk tank. The following procedure was used to select herds with elevated YCHSCC: In a first step, the 3 Swiss breeding associations (Swissherdbook, Zollikofen, Switzerland; Holstein Breeders' Federation, Posieux, Switzerland; and Swiss Brown Cattle Breeders' Federation, Zug, Switzerland) selected farms that fulfilled the following criteria: an average YCHSCC between 200,000 and 300,000 cells/mL and a minimum of 12 tested cows for each of the 11 test-days in the year 2010. Herds with fewer than 15 dairy cows, delivering milk from less than 80% of the cows to the dairy factory, with more than 2 milkings per day, or with seasonal calving, were excluded. Additionally, herds located in the canton of Ticino were excluded for logistic and language reasons. Out of these preselected dairy herds, 1,000 herds were randomly selected following stratification by breed and proportional to the number of members in the different breeding associations (Holstein Breeders' Federation n = 200, Swissherdbook n = 400, Swiss Brown Cattle Breeders'