J. Dairy Sci. 101:1–5
https://doi.org/10.3168/jds.2017-14313

Short communication: Lack of intramammary niche recolonization during a sanitation program for the contagious mastitis pathogen *Staphylococcus aureus* genotype B

C. Sartori,*† V. Perreten,‡ I. Ivanovic,* M. C. Härdi-Landerer,† and H. U. Graber*[1]
*Food Microbial Systems, Group Microbiological Safety of Foods of Animal Origin, Agroscope, 3003 Berne, Switzerland
†Institute of Agricultural Sciences (IAS), Animal Physiology Group, ETH Zurich, 8092 Zurich, Switzerland
‡Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, 3012 Bern, Switzerland

ABSTRACT

In Switzerland, sanitation programs of dairy herds infected with the contagious mastitis pathogen *Staphylococcus aureus* genotype B (GTB) have been established for several years. In recent years, *Streptococcus uberis* and non-*aureus* staphylococci have emerged as the bacteria most frequently isolated from bovine milk samples. The latter cause subclinical mastitis, and some species are more persistent or pathogenic than others. The present study aimed to investigate the developments in the intramammary colonization spectrum of 5 dairy herds undergoing a sanitation program for *Staph. aureus* GTB. We collected single-quarter milk samples aseptically from all lactating cows at 3-mo intervals during the sanitation period; after classical bacteriological analysis, MALDI-TOF mass spectrometry was used to identify the isolates to the species level. Non-*aureus* staphylococci were found to be the bacterial group most frequently occurring on the selected farms, with *Staphylococcus chromogenes* and *Staphylococcus xylosus* being predominant. The present study demonstrated that GTB-infected cows treated with antibiotics lacked systematic recolonization with other bacteria during herd sanitation for the contagious *Staph. aureus* GTB.
Key words: dairy cow, intramammary colonization, non-*aureus* staphylococci, MALDI-TOF

Short Communication

The present study is part of a sanitation study for *Staphylococcus aureus* genotype B (**GTB**; Sartori et al., 2018), which is one of the most prevalent and contagious genotypes of *Staph. aureus* circulating in Switzerland and typically causes herd problems (Fournier et al., 2008; Graber et al., 2009). All other *Staph. aureus* genotypes are responsible for sporadic infections in single quarters and cows (Fournier et al., 2008; Graber et al., 2009). Therefore, a sanitation program of *Staph. aureus* GTB-positive dairy herds was established and performed in Switzerland (Sartori et al., 2018). The progressive eradication of *Staph. aureus* GTB from infected cows may open a new intramammary biological niche, which could be recolonized by new bacteria, potentially representing a new udder health concern for the sanitized herds. In particular, non-*aureus* staphylococci (**NAS**) and *Streptococcus uberis* are among the bacteria most frequently isolated from the milk of cows with subclinical IMI (Bradley, 2002; Taponen and Pyörälä, 2009; De Visscher et al., 2015) and might therefore be possible recolonizers. Although NAS are generally considered less pathogenic than *Staph. aureus*, they can contain virulence factors, leading to problems such as toxin production, increased adhesion to the bovine mammary gland epithelium, and biofilm formation (Taponen and Pyörälä, 2009; Vanderhaeghen et al., 2014). Furthermore, some NAS species (e.g., *Staphylococcus chromogenes*, *Staphylococcus simulans*) were shown to persist in the mammary gland of infected cows causing increased SCC (Fry et al., 2014), and *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* were shown to be the species most resistant to several antibiotics typically used for the treatment of IMI in Europe, including penicillin, methicillin, macrolides, and aminoglycosides (Taponen and Pyörälä, 2009; Frey et al., 2013; Taponen et al., 2016). The use of antibiotics for the sanitation of GTB might represent, therefore, a risk of selecting for IMI-associated bacteria, particularly NAS. The aim of the present study was to investigate the developments in the bacterial intramammary colonization spectra of 5 dairy herds, which participated in a sanitation program for *Staph. aureus* GTB.

Isolates were obtained between October 2013 and September 2017 during the sanitation study performed by Sartori et al. (2018), which included 19 initially *Staph. aureus* GTB-positive dairy herds. During the sanitation study, farmers had to follow a restricted

Received December 28, 2017.
Accepted May 6, 2018.
[1]Corresponding author: hansulrich.graber@agroscope.admin.ch

number of mandatory milking procedures. The most important one was the keeping of a strict and correct milking order based on the GTB status of the cows in the herd. Only cows infected with *Staph. aureus* GTB were systematically subjected to antibiotic treatment during lactation or the dry cow period, or both, whereas IMI caused by NAS remained systematically untreated. Cows with acute or peracute mastitis were treated by the private veterinarian and were excluded from the study. The GTB-positive cows not responsive to antibiotic therapy were recommended to be culled as long as they were not pregnant.

To reduce laboratory work, 5 herds were selected for the present study, resulting in 2,640 milk samples. Aseptically collected, single-quarter milk samples of all lactating cows were bacteriologically evaluated at the first farm visit and 3 additional times at 3-mo intervals (corresponding to the samplings 1, 3, 6, and 9). The within-herd prevalence of *Staph. aureus* GTB was determined for the samplings 1, 3, 6, and 9 (Table 1) by analyzing composite milk samples of each cow by real-time quantitative PCR for the unique target gene *adlb* as described by Sartori et al. (2017). The *adlb* gene is highly sensitive and specific for *Staph. aureus* GTB both at the analytical and the diagnostic level, enabling the very specific detection of the genotype of interest (Sartori et al., 2017). Bacteriological analysis was performed according to the *Laboratory Handbook on Bovine Mastitis of the National Mastitis Council* (NMC, 1999). In brief, 10 μL of milk were plated on sheep blood agar (**BA**) plates (Biomérieux Suisse SA, Geneva, Switzerland), and bacterial cultures (morphology, hemolysis, catalase activity, Gram stain) were evaluated after 24 and 48 h of aerobic incubation at 37°C. *Corynebacterium* spp. were identified at the genus level based on typical growth after 48 h, typical morphology on BA, and catalase activity (NMC, 1999). All other bacterial species than *Corynebacterium* spp. showing a growth of at least 10^3 cfu/mL (10 cfu/10 μL) on BA were isolated and conserved in skim milk at −20°C until they were analyzed by MALDI-TOF MS as described by Dolder et al. (2017). In brief, a toothpick was used to directly smear a small amount of each colony onto the target plate (Bruker Daltonics GmbH, Bremen, Germany), followed by adding 1 μL of 70% formic acid and 1 μL of α–cyano-4-hydroxycinnamic acid-matrix (Bruker Daltonics GmbH). The MALDI-TOF MS analysis was then performed using the Microflex LT (Bruker Daltonics GmbH) using an expanded database (Dolder et al., 2017). In this expanded library, 2 species were found to be related to *Staphylococcus warneri* and to *Staphylococcus devriesei* by 16S rRNA and *rpoB* or *dnaJ* sequences and were considered as *Staph. warneri*-like and *Staph. devriesei*-like, respectively (Dolder et al., 2017). Isolates with scores ≥2.0 were identified at the species level according to the manufacturer's guidelines (www.bruker.com).

Data were expressed as counts or frequencies or presented as median, minimum, and maximum. Statistical data evaluation was performed using the Systat 13.1 software (Systat Software, San Jose, CA).

An overview of the *Staph. aureus* GTB sanitation results is provided in Table 1; during the observation period of 9 mo, herds 12, 15, and 19 were completely sanitized. Farm 11 still had 2 cows infected at sampling 9 (5% of the cows), and farm 16 could not be sanitized until the end of the sanitation program because 6 cows (21%) were still GTB-positive at sampling 9 (Table 1). Cows infected with other genotypes of *Staph. aureus* were exclusively found on farms 11 and 15 during the sanitation study (Table 1). *Corynebacterium* spp. were found on all farms at all samplings. *Streptococcus uberis* (n = 23) was isolated at low rates on all farms, whereas other *Streptococcus* spp. were found in single quarters and farms (Table 2). *Enterobacteriaceae* (n = 9) were found in single quarters on 4 farms (Table 2). Among the category others (n = 42), *Aerococcus viridans* (n = 16) was isolated at low rates on 4 farms, whereas all other species were rarely detected in single quarters and farms (Table 2).

The group of species the most frequently isolated during the present sanitation study were NAS. Out of 306 NAS isolates, 12 different species were found on the selected farms (Table 2). Five main species occurred more frequently during the observation time (Table 2), including *Staphylococcus xylosus* (n = 134), *Staph. chromogenes* (n = 82), and *Staph. haemolyticus* (n = 13), which were found on almost all farms. In contrast, *Staphylococcus sciuri* (n = 45) was only found on farms 11 and 19, with 42 isolates (93%) found on farm 11 (Table 2). Similarly, *Staph. simulans* was mainly observed on farm 19 (8 out of 9 isolates). Other NAS were also found on the different farms, but at low frequencies (Table 2). Each herd was characterized by a farm-specific intramammary colonization pattern: *Staph. xylosus* and *Staph. sciuri* were the bacteria mostly found on farm 11, particularly at 1st sampling (Table 2, Figure 1). On farm 12, *Staph. chromogenes* and *Staph. haemolyticus* were predominant, whereas *Staph. xylosus* was the main NAS species on farm 15 (Table 2). Farm 16 was characterized by the presence of *Staph. chromogenes* and *Staph. xylosus*, and the same 2 species together with *Staph. simulans* were typical for farm 19 (Table 2). Considering the total NAS quarter prevalence per farm over the 9-mo observation period, a rather constant course was observed for farms 12, 15, and 16, with median values of 6 (range = 4–7%), 10 (4–14%), and 7% (4–9%), respectively (Figure 1).