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District-wide herd sanitation and eradication of intramammary *Staphylococcus aureus* genotype B infection in dairy herds in Ticino, Switzerland

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ABSTRACT

The present study demonstrates successful herd sanitation and eradication of contagious mastitis caused by *Staphylococcus aureus* genotype B (GTB) in an entire Swiss district (Ticino) including 3,364 dairy cows from 168 farms. Herd sanitation included testing of all cows using a highly GTB-specific and sensitive real-time quantitative PCR (qPCR) assay, implementation of related on-farm measures, appropriate antibiotic therapy of GTB-positive cows, and culling of therapy-resistant animals, respectively. A treatment index was used as an objective criterion to select GTB-positive cows eligible for culling and replacement payment. Sixty-two herds (37%) were initially GTB-positive with a cow prevalence between 10% and 100% and were submitted to sanitation. Twenty months after the start of the campaign, all of these herds were free from *S. aureus* GTB, whereby 73% of them were sanitized during the first 7 mo. At the cow level, a total of 343 animals were infected. Fifty of them were immediately culled and farmers were financially compensated based on their treatment index value. The remaining 293 cows were intramammarily treated with antibiotics either during lactation using the combination of cephalexin-kanamycin or penicillin-gentamicin or at dry-off using cloxacillin. Out of these cows, 275 (93.9%) were treated successfully, meaning that their milk was twice GTB-negative by qPCR after therapy. For lactational treatment, control samples were taken ≥10 and ≥20 d after treatment, for dry-off treatment ≥14 and ≥24 d after parturition. Neither lactation number nor SCC before treatment of the cow nor the type of therapy was associated with therapeutic cure. Using data of 30 GTB-positive and 71 GTB-negative herds (1,855 observations), the effect of GTB sanitation on bulk tank milk SCC (BTSCC) was evaluated by applying a linear mixed statistical model. In the year before sanitation, BTSCC was always higher in GTB-positive than in GTB-negative herds. After the start of the campaign, BTSCC declined rapidly in the herds under GTB sanitation and achieved values that no longer differed statistically from those of GTB-free herds after only 2 mo, remaining very similar for the rest of the campaign. The farmers were very satisfied with the outcome of the campaign because all GTB-positive herds could be sanitized rapidly, sanitation was sustainable, and milk quality increased.

Key words: *Staphylococcus aureus*, cattle, mastitis, herd sanitation, cure

INTRODUCTION

Mastitis caused by *Staphylococcus aureus* is one of the most important infectious diseases in dairy cows worldwide, and it is responsible for substantial economic losses and detrimental effects on ruminant welfare (Halasa et al., 2009; Heiniger et al., 2014; Ruegg, 2017). Intramammary infections with this pathogen are usually subclinical and chronic resulting in reduced milk quality and yield as well as increased use of antibiotics (**AB**) antimicrobial agents and higher culling rates, as the therapy is often not satisfactory (Barkema et al., 2006; Halasa et al., 2009). Different bovine genotypes (**GT**) of *S. aureus* were identified during the past years and varied in their virulence, pathogenicity, and epidemiology, respectively (Fournier et al., 2008; Graber et al., 2009; Cremonesi et al., 2015).

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

In Switzerland, circulation of *S. aureus* genotype B (**GTB**) and genotype C (**GTC**; Fournier et al., 2008) is predominant. However, GTB has become a major problem for Swiss dairy farms due to its contagious nature with high SCC values and cow prevalence of up to 87% (median = 47%; Fournier et al., 2008; Graber et al., 2009). Furthermore, it poses a considerable risk for human health arising from staphylococcal food poisoning caused by its enterotoxins SEA, SED, SEJ, and SER (Fournier et al., 2008; Hummerjohann et al., 2014; Cosandey et al., 2016). Indeed, cases of food poisoning caused by *S. aureus* GTB and consumption of cheese were observed (Hummerjohann et al., 2014). In a laboratory cheese model, *S. aureus* GTB produced enterotoxins (at least SEA and SED) at scalding temperatures up to 56°C (Schwendimann et al., 2020).

Staphylococcus aureus GTB is the cattle-adapted form of *S. aureus* clonal complex (**CC**) 8 (Boss et al., 2016), which is frequently observed in infections and in the nose of humans (Sakwinska et al., 2009; Albrecht et al., 2015; Carrel et al., 2015; Bowers et al., 2018). *Staphylococcus aureus* GTB is strongly associated with dairy cattle mammary glands (Leuenberger et al., 2019); therefore, cow movement (between herds) and shared milking equipment (within herds) play key roles in its transmission (Berchtold et al., 2014; Voelk et al., 2014; van den Borne et al., 2017; Leuenberger et al., 2019). Indeed, contaminated liners are the key source for GTB transmission among cows, whereas bedding, the cow's environment, the milkers' hands and clothes, as well as flies are not relevant (Leuenberger et al., 2019). Keeping the liners GTB free by following a milking order and regular thorough cleaning of the liners and the other parts of the milking equipment after milking is, therefore, essential to interrupt the spread of the pathogen. Cow movement and shared milking equipment among cows are particularly relevant for alpine regions, because here cows from various farms are regularly sent to common alpine locations (alps) for pasturing together during the summer season. On these alps, the cows are mixed for milking, so an initially GTB-negative cow could easily be infected by the liners of a milking cluster that were previously contaminated by a GTB-positive cow.

Antibiotic therapy and vaccination against *S. aureus* in bovine mastitis are often not of satisfactory success (Gruet et al., 2001; van den Borne et al., 2010; Schukken et al., 2014; Freick et al., 2016). Reasons for the normally low treatment success using AB include the ability of *S. aureus* to form biofilms (Fox et al., 2005; Bardiau et al., 2016; Thiran et al., 2018) and its ability to live inside mammary epithelium cells and macrophages (Almeida et al., 1996; Hébert et al., 2000); these mechanisms both protect *S. aureus* from being attacked by AB. Another reason is the resistance of *S. aureus* to antimicrobials, although it is of minor relevance, at least in Switzerland and other European countries (Nemati et al., 2023).

Because of these drawbacks and because the costs caused by this pathogen are very high (Heiniger et al., 2014), a new sanitation program for controlling *S. aureus* GTB was implemented (Sartori et al., 2018a). It is based on the GTB-specific real-time quantitative PCR (**qPCR**) assay (Sartori et al., 2017) and a co-developed on-farm sanitation procedure (Sartori et al., 2018a). The qPCR test explicitly detects the *adlb* gene (coding for the adhesion-like bovine protein) as first described by Sartori et al. (2017). It was found by comparing various *S. aureus* genomes using whole genome sequencing (**WGS**) and bioinformatic methods (Sartori et al., 2017). The assay is highly sensitive and specific for this genotype and enables each GTB-positive and GTB-negative cow to be identified very reliably (Sartori et al., 2017). Furthermore, it can also be used for bulk tank milk (**BTM**) analyses detecting at least 1 GTB-positive cow among 138 negative cows (Boss et al., 2011; Sartori et al., 2017).

A previous study by Sartori et al. (2018a) included 10 dairy herds analyzed by the novel qPCR assay and 9 herds examined by classical bacteriology (Kirchhofer et al., 2011). The on-farm sanitation procedure, identical for both treatment groups, included the maintenance of a strict milking order according to the infection status of the cows, the thorough and regular cleaning of the milking equipment, the veterinary support of the farmers and, the appropriate therapy of *S. aureus* GTB-positive cows during both lactation and dry period (Sartori et al., 2018a). Furthermore, culling of treatment-resistant animals was recommended (Sartori et al., 2018a). For the qPCR-based sanitation procedure, each lactating cow was additionally tested by the qPCR assay every month and reallocated to the appropriate milking group according to the test result. Selection of the antibiotics (kanamycin for lactational treatment; cloxacillin for dry cow therapy) was primarily based on WGS of GTB-positive strains followed by bioinformatic evaluation for antibiotic resistance genes (Sartori et al., 2018a).

The study by Sartori et al. (2018a) revealed that all herds tested by qPCR (n = 10) were fully sanitized within 9 mo, whereas 3 out of the 9 bacteriologically tested herds remained not sanitized after this period. Additionally, the qPCR approach showed some further key advantages, such as the use of BTM for GTB detection at the herd level enabling herd control, or the collection of clean milk samples: udder and teats of each cow were cleaned with disposable material (single-use paper towels or fresh straw) as they were prepared for milking, and a composite milk sample was then taken immediately before attaching the milking cluster. In contrast, aseptic milk sampling (each teat is cleaned with single-use paper towels and the teat end disinfected 3 times with cotton pads soaked in

Table 1. Descriptive data on district-wide sanitation of dairy herds infected with *Staphylococcus aureus* genotype B (GTB); herd sanitation started on January 1, 2018

Item	Value
Herds involved, n/N[1] (%)	168/193 (87.0)
Herds positive for *S. aureus* GTB, n (%)	62 (36.9)
Cows involved 2018, n	3,364
Cows positive for *S. aureus* GTB, n (%)	339 (10.1)
Cows involved 2019, n	3,171
Cows positive for *S. aureus* GTB, n (%)	4 (0.1)
Cows successfully treated with antibiotic therapy, n/N (%)	275/293 (93.9)
Cows culled without therapy, n	50
Herd size 2018 (mean ± SD)	21.9 ± 16.4
Herd size 2018 (Min–Max)[2]	10–83
Milking system	
Pipe or bucket milking, n (%)	164 (97.6)
Automatic milking, n (%)	4 (2.4)

[1]n = number of dairy herds involved in the GTB sanitation project; N = total number of dairy herds present in Ticino district in 2018.

[2]Min = minimum; Max = maximum.

70% ethanol; NMC, 2017) was only feasible for herds of ~35 cows because of the high workload for sampling and for the analyses in the laboratory (Sartori et al., 2018a). Antibiotic treatment resulted in an overall healing rate at the cow level of 93% independent on cows' age, lactation number, or DIM. Furthermore, SCC decreased considerably (Sartori et al., 2018a). Finally, GTB-infected cows treated with antibiotics lacked systematic reinfection of the mammary gland with new bacteria during the sanitation process (Sartori et al., 2018b).

The study by Sartori et al. (2018a) was the pilot study that demonstrated the chosen approach to sanitize GTB-infected dairy herds by qPCR and the co-developed on-farm procedure could be implemented in the field and resulted in sustainable herd sanitation. Indeed, this approach was very successful (Sartori et al., 2018a) and was therefore used to sanitize the dairy herds of an entire Swiss district as described in the following.

MATERIALS AND METHODS

Principally, the herd sanitation procedure for *S. aureus* GTB was performed according to Sartori et al. (2018a) using the same qPCR assay (Sartori et al., 2017) and the same on-farm measures except as stated.

The Canton Ticino, the Italian-speaking district located in the South of Switzerland (area = 2,812 km^2), was selected because previous studies revealed (Boss et al., 2016; Cosandey et al., 2016; Sartori et al., 2018a) that this region has a serious problem with *S. aureus* GTB in its dairy herds. In addition, ~180 dairy herds were manageable in terms of diagnostic and personal resources. The Ticino district is a region where common alpine pasturing during the summer months (May or June until mid-September) is traditionally widespread. This means that every summer dairy cows from various farms of the district are brought together at different locations in the mountains (alps) for common grazing and the production of alpine cow cheese. However, because there is an insufficient number of cows in the Ticino district to economically manage these pastures, cows from other Swiss districts are sent there.

After awareness of the project was raised by organizing information events for farmers and veterinarians and distributing information material, dairy farmers of the Ticino district were encouraged to voluntarily take part in the program from January 2018 to December 2020. They were also informed that they would be financially compensated for cows to be culled according to an objective criterion. Only dairy farmers (n = 168; Table 1) who had signed a study participation contract and agreed to participate throughout the whole program were included in the project.

Milk Sampling for GTB Testing

Figure 1 provides an overview of the sampling procedure and times of data collection. During the campaign (2018–2020), sampling started in January and ended in April as the cows were then sent to the alps. Each lactating cow (Braunvieh or Holstein breed) was sampled every 3 to 4 wk (clean composite milk samples), at the earliest 14 d after calving. If they were qPCR negative twice in a row, they were considered GTB free and their sampling was stopped. If one sample was positive, the cow was considered GTB positive and was immediately treated (see below). Cows with lactational treatment were re-sampled at the earliest 14 d after the last therapy. If they were then twice qPCR negative in a row, they were considered GTB free and cured. If all cows of a herd were free from *S. aureus* GTB, the herd was considered GTB free.

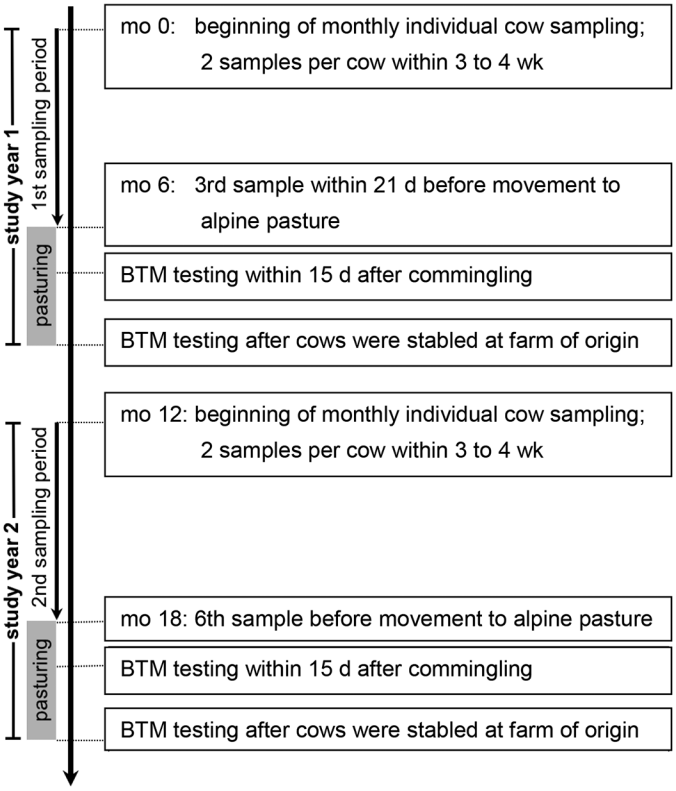

Figure 1. Study design: sampling procedure and times of data collection. BTM = bulk tank milk.

By contract, all cows of the 168 herds involved in the project were only allowed to be sent to GTB-free alps in the Ticino district. To be sure that the cows were actually free from *S. aureus* GTB, they were sampled (clean composite milk samples) once again in May or June (at maximum 21 d before the cows were sent to the alps) and analyzed by qPCR for *S. aureus* GTB. Because cows from other districts without GTB sanitation were sent to the same alps, each of these cows was tested for *S. aureus* GTB once (clean composite milk sample taken at their home farms) within 21 d before common pasturing. As a control, BTM samples of each alp were collected and tested for *S. aureus* GTB within 15 d of common pasturing. If *S. aureus* GTB was detected, each cow of an affected alp underwent individual milk sampling and analysis. The GTB-positive cows were either immediately dried off or sent back to their farm of origin. After cows had moved back from alpine pastures in fall, all herds included in the project were tested in December at their home farms using BTM samples and the qPCR assay for *S. aureus* GTB.

After the end of the GTB sanitation campaign in 2020, all cows sent to common pasturing in the Ticino district continued to be sampled (clean composite milk samples) and tested for *S. aureus* GTB. Furthermore, yearly alpine and home BTM samples continue to be analyzed for control reasons, because common alpine pasturing is the major source of *S. aureus* GTB infection (Berchtold et al., 2014; Voelk et al., 2014).

Diagnostic Procedure

Practical Milk Sampling. The BTM samples were collected as described (NMC, 2017). Furthermore, composite milk samples of cows were taken under clean conditions by instructed personnel using sterile 30-mL plastic tubes without preservatives (Sartori et al., 2017). Milk samples were stored at 4°C for a maximum of 5 d until analysis.

Real-Time Quantitative PCR. To detect *S. aureus* GTB in composite milk samples of cows or BTM, a qPCR assay for the GTB-specific *adlb* gene was used. The assay was developed by Sartori et al. (2017). It is very sensitive (3.4 cfu/100 µL) and has excellent diagnostic sensitivity (99%) and specificity (100%; Sartori et al., 2017, 2018a), so that every GTB-positive and GTB-negative cow can be identified very reliably. Furthermore, the assay can be used for BTM analyses where it detects at least 1 GTB-positive cow among 138 negative cows (Boss et al., 2011; Sartori et al., 2017).

The test was performed by a commercial diagnostic laboratory (IDEXX Diavet, Freienbach, Switzerland). In cases of analytical problems, the samples were forwarded to the Swiss reference laboratory for *S. aureus* GTB (Agroscope, Liebefeld) for definitive evaluation. To reduce analytical costs, milk from 10 cows was pooled in the commercial laboratory (1 mL of milk/cow) and then analyzed by the standard qPCR assay. In case of a positive GTB result, each sample included in the pool was analyzed separately. The dilution factor was not considered for evaluating the result of the pooled samples, because a false-negative result from the dilution was very improbable: in BTM, at least 1 GTB-positive cow in 138 negative cows can be detected (dilution 1:138) by the assay (Boss et al., 2011; Sartori et al., 2017).

Antimicrobial Resistance Testing. Antimicrobial susceptibility of *S. aureus* GTB strains which were isolated from up to 4 randomly selected cows of each positive farm was tested by the agar disk diffusion method according to the guidelines of EUCAST (http://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/) to check that the standard antimicrobial therapy (see "Herd Sanitation Procedure") can be expected to be effective.

Herd Sanitation Procedure

As performed in the study by Sartori et al. (2018a), *S. aureus* GTB-positive farms (i.e., those with ≥1 in-

fected cow) had (1) to stick to a strict milking order depending on the cows' infection status (Sartori et al., 2018a): GTB-negative animals were milked first (group 1), followed by cows with unknown GTB status forming group 2 (i.e., new animals entering the farm, cows under antibiotic mastitis therapy for *S. aureus* GTB, or cows after calving until they turned out to be either GTB-positive or twice GTB-negative, and finally group 3 including GTB-positive animals. For easier identification by the milker, the positive cows were marked with a red band fixed on one of the hind legs. Additional obligatory on-farm measures were (2) thorough cleaning of the milking equipment twice a day according to the manufacturer's guidelines, (3) cleaning the teats with single-use material, (4) postmilking teat disinfection using iodine-based products, (5) maintenance of the milking equipment by an authorized technician once a year, and (6) wearing disposable gloves during milking (newly included). Farms equipped with an automatic milking system had to conduct an additional cleaning cycle after milking each positive cow and disinfecting the liners with hot steam. For all GTB-positive farms, the sampling of cows after treatment and the on-farm measures were maintained until each cow of a herd was GTB-negative or culled (also during the summer months; see also Figure 1).

All GTB-positive cows that farmers decided to eliminate because of additional health problems or with a treatment index (i_t) below the threshold (feasible for financial compensation) were immediately culled without therapeutic intervention.

All the other GTB-positive cows received either lactational or dry-cow therapy. Cows that were ≤210 DIM received intramammary medication of either a combination of 200 mg of cefalexin and 100,000 IU of kanamycin (Ubrolexin, Boehringer Ingelheim Vetmedica GmbH) as used by Sartori et al. (2018a) or a cheaper, unevaluated combination of 250 mg of gentamicin and 2.5 million IU of procaine benzylpenicillin (Gentapen, Dr. E. Graeub AG) applied to each quarter for 5 d at 24 h intervals. Cows with >210 DIM were dried off and immediately treated after the last milking by intramammary administration of 1.28 g of benzathine cloxacillin (Orbenin Extra 1.28 g, Zoetis Schweiz GmbH) applied to each quarter. Furthermore, a dry cow prophylaxis with cloxacillin was recommended for all negative cows of GTB-positive farms. To determine a bacteriological cure, each cow was tested twice by qPCR for *S. aureus* GTB using composite quarter milk samples: the first control sample was taken ≥10 d post-treatment in lactating animals and ≥14 d postpartum in dry cows, respectively. The second control sample was taken ≥21 d after the first testing for both lactating and dry cows.

Financial Support

The farmers taking part in the GTB sanitation project were financially supported, and milk sampling, qPCR analysis for *S. aureus* GTB, and financial compensation for culled cows were paid by the project. To ensure an objective criterion for financial compensation after culling, we established the i_t based on the study by Sol et al. (1997). For each GTB-positive cow, it was calculated as $i_t = (i_l + i_c)/2$, where the lactation index (i_l) is based on the number of lactations and the SCC index (i_c) is based on the average SCC (**TSCC**) calculated from the last 3 SCC values obtained from monthly SCC recordings (for the specific i_l and i_c values see Supplemental Table S1; see Notes). When the i_t value was below a certain threshold, culling of the animal was recommended and financial compensation was provided. The threshold varied depending on the number of infected cows per herd: if the within-herd prevalence for *S. aureus* GTB was <20%, the i_t threshold was set to 0.35; for prevalence between 20% and 40% and >40%, the i_t threshold was 0.33 and 0.30, respectively; positive animals with an i_t above the threshold were treated and not financially compensated for, if slaughtered without therapeutic intervention.

Data Management

To monitor the infection status of each cow involved in the project, their GTB test results (diagnostic laboratory) and treatment data (veterinarians, farmers) were regularly transmitted to a data warehouse (run by the Federal Food Safety and Veterinary Office) and supplemented with further data from the breeding association (Braunvieh, Zug, Switzerland) including age, number of lactations, DIM, and monthly SCC (composite milk samples) of the cows. Based on these data, 2 specific, monthly updated reports were created and sent electronically to the receivers: (1) a list for the farmers with the milking order of the cows; (2) a list for the local veterinarians containing the GTB-positive cows of a farm and the description of the management thereof (lactational treatment, treatment at dry-off, or culling).

Statistics

Individual cow data including ear tag number, age, number of lactations, and DIM were transferred to Microsoft Excel (Microsoft Corporation, Redmond, WA). Statistical analyses were performed using the Systat 13.1 software (Systat Software Inc., Chicago, IL) for all analyses if not otherwise stated. Categorical data were described as frequencies, and continuous data as mean ± SD, minimum, and maximum. For rates, the nominator

and denominator were reported. All missing data were excluded from statistical analysis.

To assess whether lactation number, SCC, or the type of antibiotic treatment affected the cure of a cow, a binary logistic model was computed. For this reason, the treatment success of the individual (successfully vs. nonsuccessfully treated) was specified as a binary dependent variable. Furthermore, for each cow, the composite milk SCC of the monthly milkings recorded at the month of GTB sampling (**ICSCC**) was used and then $\log_{10}$ transformed (**log10ICSCC**). Regarding the type of treatment, a categorical variable was generated including lactational therapy either with cefalexin or kanamycin (reference), penicillin or gentamicin, or treatment at drying-off using cloxacillin. Furthermore, a categorical variable was introduced for lactation number comprising 3 levels (see also Table 2): level 0 and 1 included all cows with lactation number 1 and 2, respectively; level 2 was comprised of all cows with lactation numbers ≥3. Because the choice of the AB combination used for lactational treatment was completely farm dependent, a separate variable representing the different farms was omitted in the model to avoid a corresponding association bias.

The progress of GTB herd sanitation was assessed by a nonparametric survival analysis approach using the Kaplan-Meier method (Kaplan and Meier, 1958). To do so, the time for each herd (expressed in months) after the start of the project in January 2018 was calculated until every cow of a herd was twice GTB-negative or slaughtered (= herd sanitized). Every GTB-positive herd initially included in the study (n = 68) was followed until its sanitation was complete, meaning that no censored herds in the dataset were present. The function was computed according to Kaplan and Meier (1958) and plotted using the Systat 13.1 software (Systat Software Inc.). The observed curve was compared with a theoretical reference curve using the logrank test (Mantel, 1966), assuming for the reference that a GTB-infected herd and cow do not undergo spontaneous cure. Indeed, Sartori et al. (2018a) showed this assumption is justified: for all 21 GTB-infected herds included in the cited study, there was a history of an *S. aureus* mastitis problem both at herd and cow level that had lasted at least >1 year despite several different therapeutic interventions.

A linear mixed model was established to evaluate whether GTB infection affected milk quality (measured as SCC), and whether milk quality increased after GTB sanitation. As milk, the monthly herd BTM (**BTSCC**) was used. It was obtained from milk samples sent in for official milk quality control and was established by Suisselab AG (Zollikofen, Switzerland). This dataset was complete, because for every herd and month included in the present study the BTSCC recordings were all available. For farms whose milk was not delivered for public consumption (normally used for fattening calves), BTSCC was not available because, in this case, official milk quality control is not required by Swiss law (Swiss Administration, 2020a). The values of the BTSCC variable were $\log_{10}$ transformed. The new variable (**log10BTSCC**) served as response variable, whereas the GTB status of a herd at enrollment (GTB-negative vs. GTB-positive), observation time in months (**OT**), and their interaction served as explanatory variables. The model included herds as random intercepts. The OT started in 2017 and ended in 2020. The time between January 2017 and May 2017 was the pre-sanitation time, whereas the time between January 2018 and May 2020 reflected the time of sanitation. For each year, only the months January to May were included because afterward many herds were sent for alpine pasturing, a situation which made it no longer possible to control them during that time.

The linear mixed model addressing nonindependence of BTSCC measurement (the same farms were repeatedly sampled over time) was computed using R v.4.1.2. and the package lme4 v.1.1–28 (Bates et al., 2015). The model was evaluated using Q-Q plots of the residuals and plots of expected versus observed values. Significance was tested using the *F* Wald test with sum of errors type III, as implemented in the R package car v.3.0–12 (Fox and Weisberg, 2019). Further analysis was performed to test for differences in SCC within each month: based on the mixed model described above, the marginal means were estimated as implemented in the R package emmeans v.1.7.5 (Lenth, 2022), and the difference in marginal means between GTB-positive versus GTB-negative herds was then computed within each point in time.

Table 2. Results of the binary logistic model on the therapeutic success of the cows (successfully treated vs. nonsuccessfully treated) dependent on lactation number, log10ICSCC, and treatment[1,2]

Parameter	β ± SE	*P*-value
Intercept	−6.281 ± 2.434	0.010
Log10ICSCC	0.986 ± 0.783	0.208
Lactation 1	1.285 ± 2.839	0.651
Lactation 2	−5.571 ± 4.459	0.212
Therapy GP	−0.225 ± 0.735	0.759
Therapy C	−0.306 ± 0.619	0.621
Log10ICSCC × lactation 1	−0.374 ± 0.997	0.708
Log10ICSCC × lactation 2	1.728 ± 1.410	0.220

[1]For lactation number, the cows were grouped into 3 categories: lactation 1 (all cows in first lactation), lactation 2 (all cows in second lactation), and lactation 3 (all cows in ≥3 lactations; reference). Lactational treatment was performed with a previously evaluated combination of cefalexin and kanamycin (Ubrolexin; reference), with a new combination of penicillin and gentamicin (Gentapen; Therapy GP), or with cloxacillin at drying-off (Orbenin Extra; Therapy C). Log10ICSCC indicates the $\log_{10}$ transformation of the composite milk SCC of the monthly milkings recorded at the month of milk sampling for *Staphylococcus aureus* genotype B.

[2]β = parameter estimate.

The difference in marginal means was tested using the contrasts method implemented in the emmeans package (Lenth, 2022). Figures were plotted using the R package ggplot2 v.3.4.1. Values of $P < 0.05$ were considered significant.

RESULTS

Status of S. aureus GTB at Beginning of Campaign

Herd Level. In January 2018, at the beginning of *S. aureus* GTB sanitation, a total of 168 Ticino dairy farms were tested for *S. aureus* GTB using BTM (Table 1). Out of them, 106 farms were GTB-negative, 62 were GTB-positive, corresponding to a herd prevalence of 37% and were submitted for GTB sanitation as described above.

Cow Level. At the start of the campaign, the median GTB cow prevalence was 10.1%. Twenty-six herds (42%) showed a cow prevalence of <20%, for 15 herds (24%), the prevalence was between 20% and 40%, and for 21 herds (34%), the prevalence was > 40%. On 3 farms, all cows (100%) tested positive for *S. aureus* GTB.

In January 2018, a total of 3,364 cows were involved in the project. Out of these, 339 cows turned out to be GTB-positive during this year (overall cow prevalence = 10.1%) As decided by the farmers, 48 infected cows were immediately culled without receiving any treatment, whereas 291 (85.6%) were treated with antibiotics as described.

Status of S. aureus GTB During Campaign

Herd Level. As shown in Figure 2, all 62 initially GTB-positive herds could be sanitized within 20 mo. The number of infected herds decreased rapidly during the first 7 mo (73% of the herds were sanitized by that time), whereas a slower decline was observed for the remaining 17 herds. According to the Kaplan-Meier model, mean sanitation time was 6.9 mo (95% CI: 5.8–7.9 mo). The sanitation of 25%, 50%, 75%, and 90% herds took 4, 6, 9, and 13 mo, respectively. Accordingly, the herd prevalence dropped from initially 37% to 27.7%, 18.5%, 9.2%, and 3.7% after 4, 6, 9, and 13 mo, respectively.

Cow Level. During the campaign (in 2019), 4 additional cows (0.1%) were newly infected. Two of them were treated with antibiotics, and 2 were immediately culled. Adding these cows to the initial 339 GTB-positive animals, a total of 343 cows were GTB-positive during the campaign.

Analyzing all 343 cows, the number of GTB-positive animals decreased rapidly within the first 6 mo followed by a slower decline during the remaining 14 mo. The mean sanitation time for a cow was 5.0 mo (95% CI: 4.7–5.4 mo). The first 25% of the GTB-positive cows were GTB-free within the first 3 mo, and 50%, 75%, and 90% of the cows were sanitized within the first 4, 6, and 10 mo, respectively. The overall cow prevalence dropped from initially 10.1% to 5.1%, 2.5%, and 1.2% after 4, 6, and 9 mo, respectively.

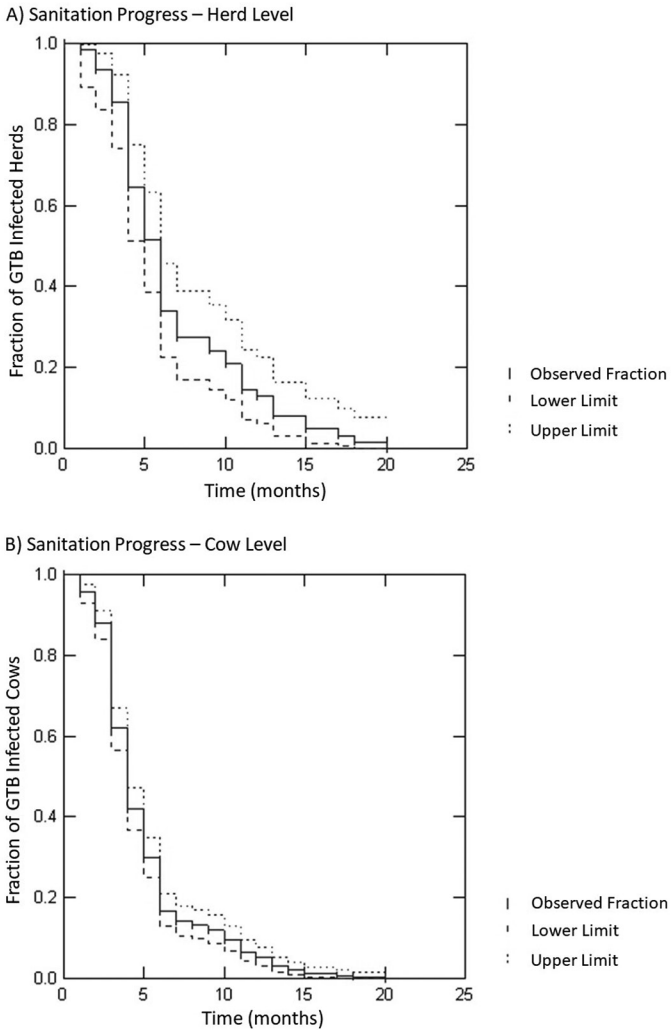

Figure 2. Progress of sanitation for *Staphylococcus aureus* genotype B (GTB). (A) Progress at the herd level. (B) Progress at the cow level. Assessment was performed by a nonparametric survival analysis approach using the Kaplan-Meier method.

Status of S. aureus GTB After Campaign Re-evaluation of all sanitized herds and all the previously GTB-free herds of the project in December 2019, 2020, and 2021 using BTM and the GTB qPCR assay revealed all herds were negative in 2019 and 2020, whereas in 2021, 2 herds were GTB-positive. Testing all lactating cows of these farms individually exposed 9 GTB-positive animals whereof 7 were then re-treated according to the standard procedure and 2 were culled.

Treatment Success

Out of 343 GTB-positive cows, 50 were immediately slaughtered without therapeutic intervention, but financially compensated for because their i_t was within the predetermined compensation range. The remaining 293 cows were treated with AB either during lactation using the combination of cephalexin-kanamycin or penicillin-gentamicin or at dry-off using cloxacillin. Out of these cows, 275 (93.9%) were treated successfully, meaning that after therapy these cows showed a GTB-negative qPCR result twice in a row. The remaining 18 cows (6.1%) with treatment failure were slaughtered and not financially compensated for.

Of all treated cows (n = 275), 55 were at their first lactation, 51 at their second, and 169 at their third or higher lactation. Neither the type of therapy nor log-10ICSCC showed any significant effect on therapeutic success (Table 2). Furthermore, no difference in treatment success was observed between older cows of ≥3 lactations (reference) and cows of first ($P = 0.708$) and second lactation ($P = 0.220$). Additionally, no significant interaction could be detected among lactation number and log10ICSCC (Table 2). The full model did not differ from the constant-only model ($P = 0.296$ and McFadden's rho squared was 0.069).

SCC in BTM

Using 30 GTB-positive and 71 GTB-negative herds for which all the necessary data were available (1,855 observations), the effect of GTB sanitation on BTSCC was evaluated using a linear mixed model. The analyses showed that log10BTSCC varied over time ($P < 0.001$; Table 3) Furthermore, a significant interaction between GTB status (infected or free) of the herd and time was observed ($P = 0.017$; Table 3). Further analysis revealed that log10BTSCC was higher in GTB-positive compared with GTB-negative herds during the year before sanitation (2017). After the start of the sanitation campaign in January 2018, log10BTSCC declined rapidly in the herds under GTB sanitation reaching a nonsignificant difference in marginal means as early as 2 mo after having started the sanitation campaign (March 2018; Table 4, Figure 2). At this point in time, 20% of the initially GTB-infected herds were fully sanitized. From March 2018 onward, the marginal means of the herds under sanitation continued to adapt (always $P > 0.05$) and ended in a value very close to the one observed for the control herds (GTB status negative) in May 2020 (Table 4, Figure 3).

Table 3. Effect of herd sanitation for *Staphylococcus aureus* genotype B (GTB) on SCC in bulk tank milk (BTSCC) delivered for commercial use[1]

Item	*F*-value	df	*P*-value
GTB status	3.54	1	0.063
Observation time	4.47	19	<0.001
GTB status × observation time	1.82	19	0.017

[1]A linear mixed model was used to assess the effect of the GTB status of a herd (infected or free) and the time of sanitation on log10BTSCC. The analysis included 30 dairy GTB-infected and 71 GTB-free herds during 2017 to 2020. Wald *F*-tests were performed to test for significance of the included variables and their interaction.

DISCUSSION

To our knowledge, this is the first report to show the eradication of *S. aureus* as a contagious mastitis pathogen in an entire district. It was made possible by comprehensive sanitation of all affected herds. Indeed, all the 62 dairy herds initially positive for *S. aureus* GTB could be sanitized within 20 mo whereby the majority (73%) of the herds had been sanitized within the first 7 mo of the campaign. At the cow level, 90% of the GTB-infected animals were GTB free within 10 mo, for the remaining 10% of the cows, another 10 mo were required. Overall, with 20 mo, the sanitation time was short although a considerable number of different farmers and veterinarians were involved. In addition, the sanitation was also sustainable, as all herds and cows remained GTB free (2018–2020) or only minimally re-infected (9 cows in 2 herds in 2021). For the infected herds, the success was accompanied by a rapid increase in milk quality and reached the same quality at the end of the campaign as for the GTB-negative herds. Importantly, a major contribution to this success was that the campaign was driven and supervised by a small team of veterinarians (L. S., M. V.) who interpreted the laboratory results and were in daily contact with their colleagues in the field and with the farmers for consulting purposes, to answer questions, and to help solving specific problems. Further major contributions were, that a proven on-farm sanitation procedure (Sartori et al., 2018a) and a robust, highly sensitive and specific GTB qPCR assay were used (Sartori et al., 2017).

Our results demonstrate that successful GTB sanitation can also be achieved for farms with an initial within-herd GTB prevalence >40% and despite additional risk factors arising from temporary common pasturing of cows originating from various farms (Voelk et al., 2014; van den Borne et al., 2017). These results confirm those of the previous field study by Sartori et al. (2018a), who successfully sanitized 10 out of 10 dairy herds using the qPCR approach for detection of positive animals, whereas only 6 out of 9 farms could be sanitized by classical bacteriology during a 9-mo period.

The qPCR assay is characterized by a very high diagnostic sensitivity (99.4%) and specificity (100%; Sartori

Table 4. Somatic cell counts expressed in cells per milliliter in bulk tank milk (BTSCC); the analysis included 30 dairy herds infected with *Staphylococcus aureus* genotype B (GTB; "infected") and 71 GTB-free herds ("free") during 2017 to 2020[1]

Date (yr–mo)	Back-transformed marginal mean infected	Back-transformed marginal mean free	Back-transformed marginal mean difference (infected vs. free)	Marginal mean difference (infected vs. free)[2]	95% CI	*P*-value
2017–01	115,543	90,530	25,013	0.106	−0.013 to 0.225	0.08
2017–02	119,689	84,479	35,209	0.151	0.032 to 0.27	0.013
2017–03	113,161	86,077	27,084	0.119	0.001 to 0.237	0.048
2017–04	144,237	91,193	53,044	0.199	0.082 to 0.316	0.001
2017–05	142,832	103,611	39,220	0.139	0.022 to 0.257	0.021
2018–01	110,638	72,438	38,199	0.184	0.065 to 0.303	0.002
2018–02	96,747	71,071	25,676	0.134	0.016 to 0.252	0.026
2018–03	94,948	80,079	14,869	0.074	−0.044 to 0.192	0.218
2018–04	102,802	86,535	16,267	0.075	−0.043 to 0.193	0.212
2018–05	107,973	95,301	12,672	0.054	−0.065 to 0.173	0.371
2019–01	105,784	91,792	13,992	0.062	−0.057 to 0.181	0.309
2019–02	105,364	82,363	23,000	0.107	−0.012 to 0.226	0.078
2019–03	94,509	89,425	5,084	0.024	−0.096 to 0.144	0.693
2019–04	98,130	90,355	7,775	0.036	−0.083 to 0.155	0.555
2019–05	114,381	96,390	17,992	0.074	−0.045 to 0.193	0.22
2020–01	114,643	101,716	12,927	0.052	−0.069 to 0.173	0.399
2020–02	105,082	99,787	5,295	0.022	−0.098 to 0.143	0.714
2020–03	99,690	95,696	3,993	0.018	−0.103 to 0.138	0.772
2020–04	103,157	101,069	2,088	0.009	−0.111 to 0.129	0.885
2020–05	121,234	119,084	2,150	0.008	−0.112 to 0.127	0.898

[1]For each year, the samples were evaluated for the months of January (e.g., January 2017–01) to May (e.g., 2017–05). The table includes back-transformed marginal means and comparisons based on the linear mixed model to assess the effect of *S. aureus* GTB herd sanitation on log10BTSCC.
[2]Difference between the marginal log10BTSCC means of GTB-infected and GTB-free dairy herds. These values resulted from the linear mixed model and were used for the post hoc analysis.

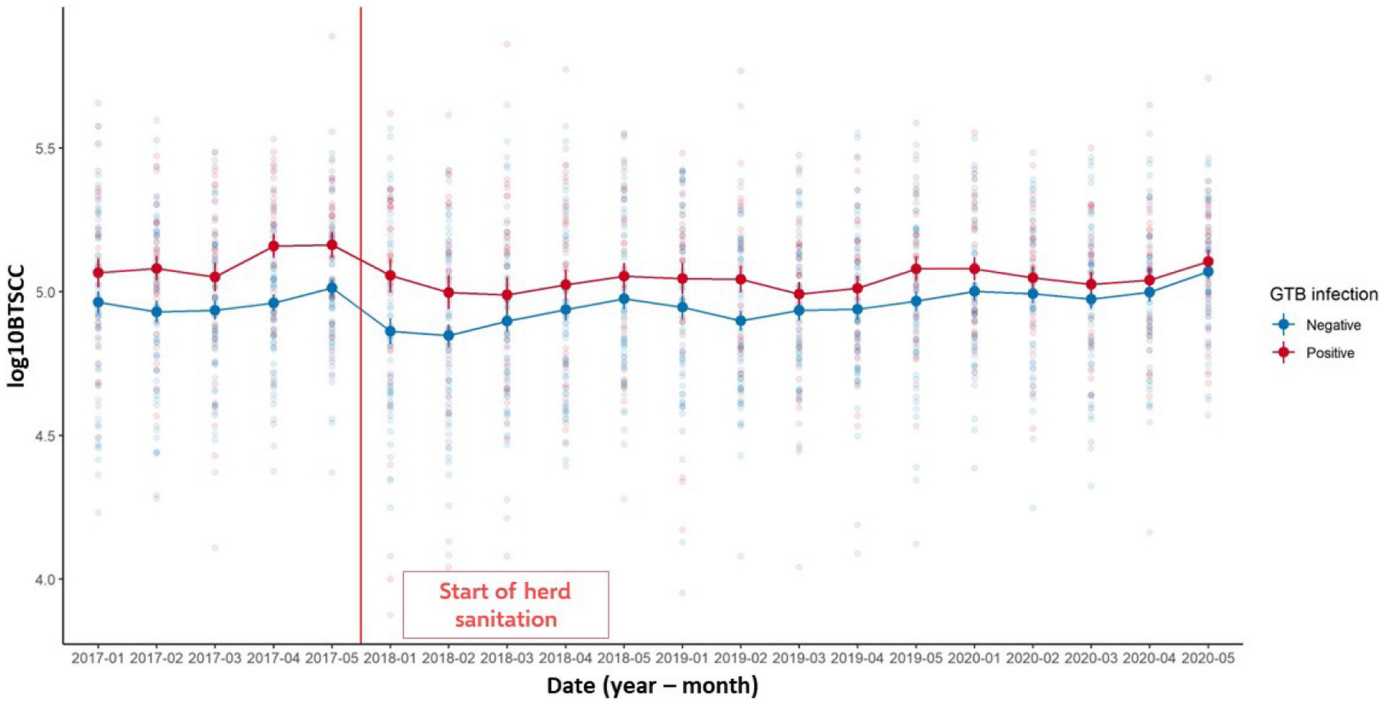

Figure 3. Effect of herd sanitation for *Staphylococcus aureus* genotype B (GTB) on SCC in bulk tank milk (BTSCC) delivered for consumption. A linear mixed model was applied using data of 30 GTB-infected and 71 GTB-free herds (1,855 observations), and $\log_{10}$ transformation of BTSCC (log10BTSCC). After 2 mo of sanitation (March 2018), the marginal means of the herds under sanitation no longer differed significantly from GTB-free herds (P always > 0.05) and remained constantly low, even after the end of sanitation in 2018. For each year, the samples were evaluated for the months of January (e.g., 2017–01) to May (e.g., 2017–05). The connected dots reflect the marginal means, and their bars indicate ±SEM. Red vertical bar: start of herd sanitation for *S. aureus* GTB.

et al., 2017), meaning that every GTB-infected cow is detected very reliably by this test whereas a GTB-negative cow is very reliably excluded from being infected. Correct identification of GTB-infected and noninfected cows is the key for an eradication program that is based on a sanitation approach (Voelk et al., 2014; Leuenberger et al., 2019). Indeed, according to this procedure, cows with a negative test result are allocated to the healthy group. If a cow with a false-negative result, however, is brought into such a group, spreading of the pathogen remains possible resulting in new infections of previously uninfected animals with the consequence that sanitation frequently fails. This is the main problem if milk testing is performed by standard plating on blood agar (Sartori et al., 2018a). In fact, by this method only a diagnostic sensitivity for *S. aureus* after single sampling of 75% is achieved (Sears et al., 1990; Studer et al., 2008), meaning that 25% of truly infected cows will show a false-negative result and will spread the disease. Actually, according to our experience, recurrent infection in the healthy group is a frequent observation in GTB-infected herds before successful sanitation and often brings the farmers close to despair. In fact, for many farmers, a GTB-infected herd not only causes professional, but also mental stress, as infection of herds caused by *S. aureus* GTB is usually characterized by a history of at least 1 to 2 years during which the farmers had tried several different treatments but none of them was capable of eradicating the disease (Sartori et al., 2018a). This leads to considerable frustration with the consequence that various farmers stop milk production and decide on a professional alternative.

In addition to its excellent diagnostic sensitivity and specificity at the cow level, the qPCR assay can also be applied for analysis of BTM samples, enabling use of this simple and inexpensive type of milk sample to detect GTB-infected herds and to control them after sanitation. With a detection limit in BTM of at least 1 GTB-positive cow among 138 negative cows (Boss et al., 2011; Sartori et al., 2017), GTB-infected herds can be very reliably identified. Furthermore, the qPCR test shows practical advantages as compared with bacterial cultivation, such as the simple collection procedure of composite milk samples (clean but not sterile), a key for sampling all cows also of large herds, the rapid formation of consistent milking groups according to the infection status of the cows, and the lower requirements for laboratory analysis concerning time and costs (Sartori et al., 2017, 2018a).

According to previous publications (Sol et al., 1997; Barkema et al., 2006), bacteriological cure of mastitis caused by *S. aureus* is associated with certain host-level factors including higher lactation number or ICSCC at treatment, and infection of multiple quarters. In the present study, dealing exclusively with one subtype of *S. aureus* (GTB/CC8), neither lactation number nor ICSCC had a significant effect on the treatment success of individual cows. Similarly, time of treatment (lactation vs. dry period) did not affect the outcome. Furthermore, no difference in treatment success was observed when lactational treatment was performed using either Ubrolexin (cefalexin + kanamycin) or Gentapen (penicillin + gentamicin). With a cure rate of 93.9% including all cows which had undergone antibiotic therapy, the success was very high and was approximately identical (93%) to the one observed by Sartori et al. (2018a), even though the present study included many more herds, cows, and veterinarians than the previous one. Importantly, as defined by Sartori et al. (2018a), cure was considered as 2 consecutive negative results obtained by the GTB qPCR assay. With the test's diagnostic specificity of 100% (95% CI = ±2%; Sartori et al., 2017) and 2 samplings in a row, the probability is close to zero that a cow was wrongly considered to be cured. Because no germicides were used for teat disinfection before sampling it cannot be ruled out, however, that some cows were wrongly considered to suffer from GTB IMI because their teats were colonized by the pathogen but the mammary gland was actually not infected. These IMI false positives, although healthy, were treated too and may have caused, therefore, some inflation of the described cure rate. However, reevaluation of the data by Sartori et al. (2018a) revealed that all GTB-positive cows remained GTB positive in consecutive samplings as long as they were not treated (resampling GTB-positive cows was omitted in the present study to save costs). Constant detection of *S. aureus* GTB in the milk of the same cow over weeks is a clear indicator that IMI was the source of the pathogen in these cases. Taken together, the observed cure rate of 93.9% likely reflects the real rate.

Compared with previous studies with a reported median cure rate of ~30% (Gruet et al., 2001), the observed rate of antibiotic therapy for *S. aureus* is very high (Sol et al., 1997; Gruet et al., 2001; Barkema et al., 2006). Several reasons may have contributed to this success: as performed by Sartori et al. (2018a), antimicrobial treatment was extended to 5 d in lactating cows because prolonged therapy enhances the cure rate of subclinical IMI caused by *S. aureus* (Barkema et al., 2006). In addition, as in the previous study, antibiotics were administered to all 4 quarters because *S. aureus* GTB commonly infects 2 or more quarters of a cow (Fournier et al., 2008). Furthermore, resistance to aminoglycoside antibiotics (e.g., kanamycin, gentamicin) in Swiss mastitis-associated *S. aureus* isolates is rare with a resistance rate of 1.7% (Overesch, Stephan and Perreten, 2013) and 0% (Käppeli et al., 2019), values that have recently been confirmed by a European study showing 0.5% aminoglycoside resistant *S. aureus* isolates (Nemati et al., 2023). Finally, we are gaining more and more evidence that the success rate

of antibiotic treatment of *S. aureus* is genotype dependent. Indeed, the cure rate is very high for *S. aureus* GTB as shown in the present study and in the one by Sartori et al. (2018a), but it seems to be considerably lower for GTC and GTR (own clinical experience). However, this is not because of increased resistance rates for these GT to penicillin and aminoglycoside antibiotics as shown by Nemati et al. (2023). Rather, these GT may differ from GTB by their biological properties. Indeed, recent studies using genomic, transcriptomic, and secretomic analyses demonstrated that biological differences among GT actually exist (Capra et al., 2017; Addis et al., 2022; Di Mauro et al., 2023). Further investigations, however, are necessary to confirm the hypothesis about different reactions of GT against AB in vivo.

The present study demonstrates that sanitation of *S. aureus* GTB-infected herds not only led to GTB eradication, but also to increased udder health and milk quality as BTSCC of infected herds, starting from an increased level, dropped within 2 mo of sanitation to a level that did no longer differ significantly from the GTB-negative control herds. The BTSCC remained low and stable even after 1 yr after sanitation (2020), demonstrating the sustainability of the sanitation campaign. Furthermore, it was also possible to decrease considerably the use of antibiotics during common pasturing on the alps after successful sanitation ($P = 0.004$; Vaccani et al., 2022), the location with the highest risk for a cow to get infected by *S. aureus* GTB (Berchtold et al., 2014; Voelk et al., 2014; van den Borne et al., 2017). For GTB-positive herds, the use of antibiotics for mastitis treatment increased during the sanitation and decreased afterward to the initial amount whereby, in tendency, it was even lower ($P = 0.068$) than the one used for the control herds (Vaccani et al., 2022).

To ensure an objective selection of GTB-positive cows eligible for culling and financial compensation, a treatment index (i_t) was established. It was based on the study by Sol et al. (1997) demonstrating that increased parity and SCC at the time of treatment impaired the success of antibiotic therapy. Contrary to our expectations, however, neither lactation number nor TSCC had a significant effect on the treatment success meaning that this index was basically inappropriate as a selection criterion. Nevertheless, it was an objective and reproducible evaluation tool to justify official financial compensation. Retrospectively seen, however, this payment was probably not necessary because with a treatment success rate of 93.3%, the majority of the culled cows might also have been cured successfully. Importantly, this study demonstrates that objective criteria associated with the outcome of therapy are required with respect to economic and animal welfare issues.

Additional Aspects

Sanitation of *S. aureus* GTB not only increased udder health und milk quality in the Ticino district, but it also improved food safety in raw milk cheese as demonstrated in the official governmental report (www4.ti.ch/fileadmin/DSS/DSP/LC/lcinforma/Rapportini/2022/Alpeggi_2022.pdf). In the frame of regular quality controls of dairy products required by Swiss law (Swiss Administration, 2020b), the presence of coagulase-positive staphylococci (**CPS**) in samples of curd prepared from raw milk was measured every year, following a standardized protocol (Swiss Administration, 2020a). According to these analyses, the percentage of samples with CPS content conforming to Swiss law (<10,000 cfu/g) increased from ~58% (mean over years) before the start of the sanitation for *S. aureus* GTB to ~80% after sanitation in 2018. Since then, it has remained largely constant.

In March 2022, a questionnaire had been sent to all farmers who had participated in the GTB sanitation project in the Ticino district having also included those whose herds had tested negative at the start of the project (Supplemental Tables S2 and S3; see Notes). The questionnaire had comprised various questions dealing among others with the reasons for participation and success of the project. The main reason was to improve milk quality, followed by eradication of the disease to solve an old problem, reduction of antibiotics, and improvement of food safety. Furthermore, 97% of the farmers stated that they would again participate in a GTB sanitation project if necessary in the future, demonstrating that the farmers were very pleased with the benefits achieved for their own farms and for their region.

Generalizability of Results

The described sanitation procedure with qPCR assay and related on-farm measures can be taken over directly to sanitize all areas where *S. aureus* GTB is observed. This is particularly true for other Swiss regions, but also for regions in Austria, France, Germany, and Italy, where *S. aureus* GTB was also found (Cremonesi et al., 2015; Cosandey et al., 2016). According to Monistero et al. (2018), however, other contagious genotypes exist whose biological and genetic properties may differ from those of *S. aureus* GTB. In this case, the studies by Sartori et al. (2017, 2018a) need to be repeated. In particular, a novel qPCR assay specific for the particular genotype needs to be developed as conventional bacteriologic methods are no longer suitable to deal with large numbers of herds and cows (Sartori et al. 2018a). Furthermore, a simple and elegant way of using BTM for a first herd evaluation and control after sanitation is not available.

CONCLUSIONS

The present study included a total of 168 dairy herds of the Ticino district comprising 62 herds being initially positive for *S. aureus* GTB in BTM. The only genotype causing staphylococcal contagious mastitis *S. aureus* in Switzerland is GTB. Based on our previously developed qPCR assay with its very high specificity and sensitivity for *S. aureus* GTB and its associated sanitation procedure in the field, all 62 herds could be sustainably sanitized from this pathogen within 20 mo. With 93.3% of all cows having undergone antibiotic therapy, the cure rate was very high. Furthermore, GTB sanitation was associated with a fast reduction of SCC in delivered BTM and, therefore, with increased milk quality, with reduced application of AB for mastitis treatment, improved food safety, and very pleased study participants. With the presented approach, successful herd sanitation and sustainable eradication of contagious mastitis caused by *S. aureus* GTB can be expanded to herds of a whole district.

NOTES

The authors thank all the farmers for their participation, as well as the Swiss Federal Office for Agriculture (FOAG, Bern, Switzerland), Swiss Federal Food Safety and Veterinary Office (FSVO, Bern, Switzerland), the Canton Ticino Milk Producers Federation (FTPL, Cadenazzo, Switzerland), and the Swiss Milk Producers (SMP, Bern, Switzerland), for financial support. Furthermore, the authors thank D. Dietrich and L. A. Rojas Mora, Competence Center of SAKK, Bern, Switzerland, for statistical support and computation of the mixed linear model to assess the effect of herd sanitation for *Staphylococcus aureus* genotype B on milk quality. No ethical approval about animal welfare was required due to the absence of animal discomfort during the sample collection, as approved by T. V. and L. N. B., responsible for animal welfare in Ticino. The participating farmers were fully informed about the GTB sanitation project before it was started, voluntarily participated in the project, and signed a contract regulating all steps of the project they were involved in. By doing so, they also agreed that their cows were regularly sampled and agreed to provide project-related clinical and laboratory data about their cows. Supplemental material for this article is available at https://doi.org/10.17632/ggfrmygmpp.1. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: β = variable estimate; AB = antibiotics; BTM = bulk tank milk; BTSCC = bulk tank milk SCC; GT = genotype; GTB = *S. aureus* genotype B; GTC = *S. aureus* genotype C; i_c = SCC index; ICSCC = composite milk SCC of the monthly milkings recorded at the month of GTB sampling; i_l = lactation index; i_t = treatment index; log10BTSCC = BTSCC $\log_{10}$ transformed; log10ICSCC = ICSCC $\log_{10}$ transformed; Max = maximum; Min = minimum; OT = observation time; qPCR = real-time quantitative PCR; TSCC = average composite milk SCC of a cow calculated from the last 3 SCC values obtained from monthly SCC recordings; WGS = whole genome sequencing.

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