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A longitudinal study on transmission of *Staphylococcus aureus* genotype B in Swiss communal dairy herds

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ABSTRACT

Staphylococcus aureus is a common mastitis causing pathogen of dairy cattle. Several *S. aureus* genotypes exist, of which genotype B (GTB) is highly prevalent in Swiss dairy herds. Dairy farming in mountainous regions of Switzerland is characterised by the movement of dairy cattle to communal pasture-based operations at higher altitudes. Cows from different herds of origin share pastures and milking equipment for a period of 2 to 3 months during summer. The aim of this longitudinal observational study was to quantify transmission of *S. aureus* GTB in communal dairy operations. Cows ($n = 551$) belonging to 7 communal operations were sampled at the beginning and end of the communal period. Transmission parameter β was estimated using a Susceptible-Infectious-Susceptible (SIS) model. The basic reproduction ratio R_0 was subsequently derived using previously published information about the duration of infection. Mean transmission parameter β was estimated to be 0.0232 (95% CI: 0.0197–0.0274). R_0 was 2.6 (95% CI: 2.2–3.0), indicating that *S. aureus* GTB is capable of causing major outbreaks in Swiss communal dairy operations. This study emphasized the contagious behaviour of *S. aureus* GTB. Mastitis management in communal dairy operations should be optimized to reduce *S. aureus* GTB transmission between cows and back to their herds of origin.

1. Introduction

Staphylococcus aureus is a mastitis causing pathogen that remains prevalent in dairy herds worldwide despite decades of research and control programs. A wide spectrum of *S. aureus* strains has been reported to cause intramammary infections (IMI). In Switzerland, 17 genotypes were identified in a convenience sample of milk samples (Fournier et al., 2008). *S. aureus* genotype B (GTB) and C were most commonly identified (Fournier et al., 2008). In a follow up study, *S. aureus* GTB was associated with a high within-herd prevalence whereas *S. aureus* genotype C was associated with a low within-herd prevalence (Graber et al., 2009). *S. aureus* GTB is the cattle-adapted form of clonal complex 8 (Boss et al., 2016) which is a well-known clonal complex causing infections in multiple species (van den Borne et al., 2010a), including humans (Deurenberg and Stobberingh, 2008). *S. aureus* GTB made the human-to-bovine jump approximately 38 years ago (Boss et al., 2016) and is now mainly detected in dairy herds located in central Europe (Cosandey et al., 2016).

During the summer months dairy farming in mountainous regions of Switzerland is characterised by the commingling of cows from several different herds of origin in large communal pasture-based dairy operations. Cows are brought together on dairy operations at higher altitudes where they share pastures, milking equipment and housing facilities for a period of 2 to 3 months. At the end of the communal period, cows are transported back to their herds of origin at lower altitudes. Hereafter, these high altitude summer dairy operations are referred to as communal operations. Most cows are transported to a communal operation together on a single date. However, some cows may arrive later or leave earlier, with the latter occurring more frequently. Seasonal calving is common for dairy herds located in mountainous regions of Switzerland and most cows on communal operations are approaching the dry-off period when they return to their herds of origin. Farmers transport cows that need to be dried off or that are severely diseased cows back to their herds of origin earlier. This

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commingling of cows from different herds of origin contributes to the spread of contagious pathogens (Presi et al., 2011; Spergser et al., 2013) but quantitative data about *S. aureus* GTB transmission in communal operations is lacking while this would add to the development of intervention strategies.

The aim of this study was to quantify transmission of *S. aureus* GTB among Swiss dairy cows in communal pasture-based operations.

2. Material and methods

2.1. Data collection and description of communal operations

Data were obtained during a previously described longitudinal study in which 9 communal dairy operations (labelled A to I) were selected by convenience. For a detailed description of the herd selection and sampling procedure see Voelk et al. (2014). Briefly, all dairy cows in these operations were sampled at the beginning and the end of the communal period during the summer of 2012. Clean composite milk samples were taken from all cows by trained study personnel at the first or second milking after they arrived at their communal operation. Milk sampling was performed again 3 to 7 days before transporting cows back to their herds of origin at the end of the communal period. After receiving a training, farmers were instructed to collect clean composite milk samples from cows that arrived late or left the communal operation early to ensure that all cows were sampled.

Cows were milked twice daily and premilking udder preparation consisted of the cleaning of udders with paper towels or wood shavings and fore-stripping. Milking gloves were not used. All milking machines were serviced before the communal period. Cows were not vaccinated against any mastitis pathogens. Post-milking teat disinfection (PMTD) was applied in all 9 communal operations. Cows with clinical mastitis were treated with intramammary antimicrobials and remained on the communal operation but were not sampled for *S. aureus* GTB determination. Treated cows were milked separately using a different milking cluster.

2.2. Laboratory analysis

Identification of *S. aureus* GTB in collected composite milk samples was performed as described previously (Voelk et al., 2014). In short, milk samples were diluted 1:10 with Chapman medium and incubated at 37 °C for 18 h. After this enrichment step, the bacterial culture was processed using the mericon DNA Bacteria Plus Kit (Qiagen Instruments AG, Hombrechtikon, Switzerland) for the isolation of DNA from bacteria. Real-time PCR was performed to detect the presence of three *S. aureus* GTB specific genes. *LukEB*, *sea* and *sed* code for the GTB-typical polymorphism within the *lukE* gene and for the two staphylococcal enterotoxins A and D, respectively. Diagnostic sensitivity and specificity of this test are reported to be close to 100% (Boss et al., 2011; Syring et al., 2012).

2.3. Statistical analysis

A Susceptible-Infectious-Susceptible (*SIS*) model was used to quantify cow level transmission of *S. aureus* GTB within communal dairy operations (Lam et al., 1996; Zadoks et al., 2002; Barlow et al., 2013; Schukken et al., 2014). Cows that tested positive for *S. aureus* GTB classified as being infectious (I), and *S. aureus* GTB-negative cows were classified as susceptible (S). Cows could not become immune and were assumed to mix randomly. Segregation of cows with deprived udder health (i.e., milking them last or with a separate milking cluster) was not practiced on communal operations (Voelk et al., 2014). Susceptible cows were all assumed to be equally susceptible and infectious cows were assumed to be equally infectious throughout the infectious period. New *S. aureus* GTB IMI were assumed to have occurred halfway through the study period. Cows that left the communal operation earlier and were not sampled at the end of the summer period were not included in the analysis. In some cases, cows left the communal operation earlier (or arrived there later) and were sampled by the herdsmen, but did not have the date of sampling recorded. These cows were assumed to stay, on average, for two-third of the time the majority of cows remained on the communal operation.

Dynamics of *S. aureus* GTB IMI can be quantified by transmission parameter β which represents the number of secondary IMI resulting from one *S. aureus* GTB infectious cow per unit of time (i.e., day in this study). In a population of size N (where $N = S + I$), the number of secondary cases (C) per time interval (Δt) depends on the number of susceptible (S) animals at the start of the interval, the average number of infectious (I) animals per interval, and transmission parameter β. Transmission parameter β can then be estimated as a function of I, S, C, N, and Δt. Data were analysed using a generalized linear model (PROC GENMOD) in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with a complementary log–log link function, C as the number of new cases and S as the number of trials in the binomial process. The term log $((I/N) \text{ x } \Delta t)$ was used as an offset, with I denoting the average number of infectious cows, N the total number of cows at the start of the interval, and Δt the mean duration of the sampling interval for each communal operation. Because the number of new cases C at the end of each interval can be estimated using $1 - e^{-\beta (I/N) \Delta t}$, the linear relationship for the statistical model equals (Velthuis et al., 2003; Schouten et al., 2009):

$$c \log \log \left(\frac{C}{S} \right) = \log \beta + \log \left(\left(\frac{I}{N} \right) \cdot \Delta t \right)$$

Exponentiation of logβ (i.e., the intercept of the statistical model) yields the transmission parameter β. The deviance dispersion parameter was forced to one because some underdispersion was present (i.e., the deviance dispersion parameter was 0.78). Evaluation of model fit gave no reasons for concern; both the Pearson and deviance χ^2 test were non-significant.

The reproduction ratio (R_0) is the average number of secondary infections arising from one infected individual during its entire infectious period in a fully susceptible population (Keeling and Rohani, 2008; Vynnycky and White, 2010). R_0 was estimated by multiplying β with 110, the arithmetic mean duration of *S. aureus* IMI (in days) reported in literature (Barlow et al., 2009). Significance of log(I), when added to the model with log($\Delta t/N$) being the offset (Lam et al., 1996; Zadoks et al., 2001; Schukken et al., 2014), was calculated in order to test the hypothesis that *S. aureus* GTB depends on the number of *S. aureus* GTB infected cows (and thus can be considered a contagious genotype).

3. Results

Communal operations C and G were *S. aureus* GTB-negative at first sampling (Voelk et al., 2014) and thus excluded from transmission parameter estimation. A further 82 cows were excluded because they were not sampled when they arrived later (n = 8) or left the communal operation earlier (n = 74). The final total study population consisted of 551 dairy cows originating from 83 individual herds of origin, and transported to 7 communal operations for the summer of 2012. Of those 551 cows, 10 late arriving cows did not have their arrival date recorded and 77 cows that left early did not have their leaving date recorded.

The number of susceptible (S), infectious (I) and new cases of *S. aureus* GTB (C) and the average interval duration per communal pasture are presented in Table 1. A total of 144 new *S. aureus* GTB cases were observed. Transmission parameter β was estimated to be 0.0232 (95% CI: 0.0200–0.0269) given a model intercept estimate