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Intramammary infections with the contagious *Staphylococcus aureus* genotype B in Swiss dairy cows are associated with low prevalence of coagulase-negative staphylococci and *Streptococcus* spp.

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ABSTRACT

The association between the contagious *Staphylococcus aureus* genotype B (GTB) and the presence of coagulase-negative staphylococci (CNS) and *Streptococcus* spp. (non-agalactiae streptococci), was investigated, and the identification of problem herds without genotyping was evaluated. Milk samples from 10 herds with *Staph. aureus* GTB herd problems (PH cases) were compared with samples from 19 herds with at least one *Staph. aureus* isolate of non-B genotype (CH cases). All samples were bacteriologically analysed and *Staph. aureus* genotyping carried out using a ribosomal spacer-PCR.

Cow and quarter prevalences of *Staph. aureus*, CNS and *Streptococcus* spp. differed significantly between PH and CH groups. PH cases were highly associated with decreased cow prevalences of CNS and *Streptococcus* spp. These altered prevalences also contributed significantly to the identification of problem herds without resorting to genotyping. Common herd-level risk factors did not explain the difference between the prevalences in PH and CH cases.

Introduction

Staphylococcus aureus is globally one of the most important pathogens causing contagious mastitis (Schällibaum, 1999; Zecconi et al., 2005) and is of great economic importance (IDF, 2005; Sears and McCarthy, 2003; Seegers et al., 2003). *Staph. aureus* is often referred to as a contagious pathogen, because it is commonly spread from infected cows to other non-infected cows at milking (Bartlett and Miller, 1993; Fox and Gay, 1993; Sears and McCarthy, 2003).

A recent descriptive study from Switzerland (Fournier et al., 2008) isolated *Staph. aureus* from bovine intramammary infections (IMI) and found that the organism in that country is a genetically heterogeneous group. A total of 17 genotypes were detected by ribosomal spacer (RS)-PCR from 101 epidemiologically independent isolates. Two of the genotypes, B (GTB) and C (GTC) were numerous (together accounting for 80.2% of the isolates), whereas the other 15 genotypes (GTOG) were more rarely detected (1–4% of the isolates).

The different genotypes were highly associated with their virulence gene patterns (VGP). The corresponding patterns were obtained by (1) PCR-testing for the presence of the staphylococcal enterotoxin genes *sea* to *sej* and the *tst* gene (toxic shock syndrome toxin-1; TSST-1), (2) evaluation for polymorphisms of the protein A (*spa*) and the coagulase (*coa*) genes (PCR), and (3) by searching for restriction fragment length polymorphism (RFLP) of the leukotoxin E gene (*lukE*). In short, GTB was characterised by the presence of the *sea*, *sed* and *sej* genes, a long x-region of *spa*, and 3 *lukE* fragments, whereas GTC was positive for *sec*, *seg*, and *tst*, a short x-region of *spa*, and 2 *lukE* fragments.

The GTOG were heterogeneous in their VGP (Fournier et al., 2008). Likewise, the authors found differences in the prevalence of IMI among the genotypes. If GTB was isolated, between 22.2% and 64.7% (median 47.2%) of the cows of a herd had IMI with this genotype and in 49% of the cows, more than one quarter was infected. In contrast, GTC had a cow prevalence between 2.6% and 33.3% (median 7.1%), and GTOG had a prevalence of between 2.9% and 7.1% (median 6.3%). GTC and GTOG were found at most in three cows in one herd, and in all cases, only one quarter of a cow's udder was infected.

Similar results were reported by Graber et al. (2009), who selected and compared herds with a quarter prevalence of *Staph. aureus* based on routine bacteriology of herds $\geqslant$10% and herds with a quarter prevalence of *Staph. aureus* of 0% to <10%. They reported that GTB was characterised by a high cow prevalence (between 18.2% and 87.5%, median 44.0%) and quarter (between 11.3% and 45.3%, median 25.2%), whilst GTC and GTOG affected individual cows (prevalence range between 4% and 33.3%, median 9.1%) and single quarters (prevalence range between 1.0% and 8.3%, median 2.5%).

Risk factors represent areas of interest for the prevention and control of diseases. Characteristics that increase the risk of

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infection can be identified by risk factors studies. It was found that milking order, milking technique, type of housing, teat dipping and dry cow therapy can affect the prevalence of mastitis pathogens (Bartlett and Miller, 1993; Dargent-Molina et al., 1988; Erskine et al., 1987; Hutton et al., 1990; Moret-Stalder et al., 2009).

The aim of the present study was to evaluate the association between *Staph. aureus* GTB and other mastitis-related pathogens, namely, coagulase-negative staphylococci (CNS), *Streptococcus* spp., and *Corynebacterium* spp., in Swiss dairy herds with and without *Staph. aureus* problems. In addition, we analysed potential risk factors for the presence of different mastitis pathogens at the herd level and aimed to develop a discriminant analysis to allow the correct identification of *Staph. aureus* problem herds, without genotyping the *Staph. aureus* strains involved.

Materials and methods

Herd selection

The study used the same herds as Graber et al. (2009). Herds were defined as problem herds (case cohorts, PH), if the quarter prevalence of *Staph. aureus* based on routine bacteriology (10 μL aliquot) was ⩾10%. Herds with a quarter prevalence of *Staph. aureus* between >0% and <10% (herds with sporadic IMI) were defined as control cohorts (CH). All herds analysed were spread over the western half of Switzerland (ca. 20,000 km^2). Herd size and cow breed were not used as criteria for selection. A total of 10 PH (265 cows, 1057 quarters) and 19 CH (257 cows, 1021 quarters) were included.

Sample and on-farm data collection

In each of the 29 herds investigated, all lactating cows (n = 522) were evaluated for udder health, comprising clinical examination of udder, teats and visual milk inspection. After forestripping, milk samples were taken aseptically for bacteriological testing later. During transport to the laboratory, the samples were kept at 4 °C and analysed for somatic cell count (SCC) within 24 h. Samples for bacteriological and PCR analysis were stored at −20 °C until further use.

During the respective farm visits, questionnaires were used to collect information about the type of housing (free stall/tie stall), biosecurity (closed/open herd), lying surface (rubber mat/concrete), bedding (short straw/long straw), milking system (bucket milking system/high line system/low line system), milking order (yes/no), post-dipping teat disinfection (yes/no) and antibiotic dry-cow treatment (all cows/selected cows/not performed).

Bacterial identification

The identification of *Staph. aureus* and other mastitis pathogens such as CNS, *Streptococcus* spp., *Strep. agalactiae* or *Corynebacterium* spp. was according to the guidelines of the National Mastitis Council (NMC, 1999), which include colony morphology, catalase test, Gram stain, further biochemical properties, CAMP-test and detection of haemolysis. All 205 isolates of *Staph. aureus* were checked by PCR for the presence of the *nuc* gene, which codes for thermonuclease and is known to be highly specific for *Staph. aureus* (Brakstad et al., 1992; Graber et al., 2007). Gram-positive, catalase-positive cocci lacking the *nuc* gene were recorded as CNS, while all the *nuc*-positive isolates were subjected to genotype analysis (described in detail in Graber et al. (2009)).

Analysis of somatic cells

Quarter SCC were analysed individually by a Fossomatic cell counter 5000 basic (Foss). Before analysis, the milk samples were pre-warmed at 37 °C for 10 min. Cow somatic cell count (SCC) was calculated as the arithmetic mean of the quarter cell counts (Schukken et al., 1999).

Statistical analysis

Data were expressed as mean ± standard deviation (SD), or as median with minimum and maximum. Outliers were included in the analyses to present the data as they were actually observed. Somatic cell counts were expressed as $\log_{10}$ (cells/mL). Means were compared by Student's t test using the corresponding variances of each analysis. Comparison of median was performed by the Mann–Whitney U-test. Variabilities were compared using Mood's rank dispersion test (Mood, 1954). Binary logistic regression was applied for risk factor analysis. For this purpose, the eight risk factors (categorical variables) as defined in the questionnaire (see below) were used. The full model was then compared to the constants-only model by the Likelihood-Ratio test. For herd identification, a discriminant analysis was performed using the binary logistic regression approach. Problem and control herds were discriminated by including, for each herd, the cow prevalence of CNS (CNSPrevQ), the cow prevalence of *Streptococcus* spp. (StreptPrevQ) (continuous) and the SCC150 value (1: cow prevalence <30%; 0: cow prevalence ⩾30%) whereby SCC150 signifies the prevalence of cows with four-quarter SCC > 150,000 cells/mL as described by Graber et al. (2009). All the statistical analyses, except the Mood-Test (performed manually), were computed by the Systat 12 software (Systat Software Inc). Significance was defined at values of $P < 0.05$. Multiple testing was performed by P value adjustment according to Holm (1979).

Results

Mastitis pathogens

Staph. aureus was observed in all PH and CH. The frequency of its occurrence, however, differed markedly between the two groups (Table 1, Fig. 1), as was expected from the design of the study. In the 10 PH, 127/265 cows (47.9%) were positive in one or more quarter. In seven PH, GTB was the only genotype observed, while in the three remaining herds, GTB was the predominant genotype, but IMI with GTC and GTOG were additionally present in two and three quarters, respectively.

In the CH, 25/257 cows were infected with *Staph. aureus*. GTC was detected in 21 quarters, and GTOG in six. GTB was not observed. In 14 CH, only one cow was infected; in four CH, two cows and in one CH three cows were infected. The median quarter prevalences of *Staph. aureus* clearly differed between PH and CH ($P < 0.001$) (Table 1, Fig. 1). The median prevalences of CNS were significantly higher in CH compared with PH at the cow and at the quarter levels (both $P < 0.001$) (Table 1, Fig. 1). *Strep. agalactiae* was not observed in any herd. For the remaining streptococci, the median prevalence was significantly higher in CH than in PH at both the cow ($P = 0.041$) and quarter ($P = 0.046$) levels (Table 1, Fig. 1). Cow and quarter level prevalences for *Corynebacterium* spp. were similar in PH and CH, but the variability in prevalence was significantly higher in CH than in PH ($P = 0.016$) (Table 1, Fig. 1).

Somatic cell count

The means of $\log_{10}$ (SCC) clearly differed between CH and PH ($P < 0.001$). For the CH, the mean composite $\log_{10}$ (SCC) value

Table 1
Cow and quarter prevalences of *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Streptococcus* spp. (except *Streptococcus agalactiae*) and *Corynebacterium* spp. observed in control (CH) and problem herds (PH).

Species	Cow prevalence (%)		Quarter prevalence (%)	
	CH	PH	CH	PH
S. aureus				
Median	9.1[a]	44.0	2.5[a]	25.2
Maximum	33.3	87.5	11.1	45.3
Minimum	4.0	18.2	1.0	9.8
CNS				
Median	75[a]	27.5	43.8[a]	11.4
Maximum	100	56.4	67.8	17.4
Minimum	26.7	4.3	6.7	2.2
Streptococcus spp.				
Median	33.3[b]	15.6	10.4[c]	4.4
Maximum	53.3	37.5	25.0	12.5
Minimum	0.0	2.9	0.0	0.7
Corynebacterium spp.				
Median	62.5	54.4	22.9	25.1
Maximum	100	81.8	79.5	45.5
Minimum	0.0	30.4	0.0	10.9

[a] $P < 0.001$.
[b] $P = 0.041$.
[c] $P = 0.046$.