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1        **Bacteriological and molecular investigations of *Staphylococcus aureus* in dairy goats**

2

3

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15       Running title: *Staphylococcus aureus* in dairy goats

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18

1 **Abstract**

2 In order to investigate reservoirs of *Staphylococcus aureus* in dairy goats, samples for  
3 bacteriological analyses were collected from 7 herds. *S. aureus* was detected in 353 (6.2%) of  
4 5671 milk samples, 53 (9.9%) of 535 teat skin swabs, 392 (68.9%) of 569 nasal swabs and in  
5 180 (31.6%) of 569 vaginal swabs. Vaginal swabs were more often *S. aureus*-positive after  
6 kidding (44.9%) than before drying off (19.1%), while nasal swabs were more often positive  
7 before drying off (75.6%) than after kidding (62.0%). Retrieved *S. aureus* isolates were  
8 compared by pulsed-field gel electrophoresis (PFGE), and selected isolates were tested for  
9 enterotoxin genes (*se*) by PCR. By PFGE, 505 *S. aureus* isolates were divided into 33  
10 pulsotypes (PTs). The 5 most prevalent PTs included 73.3% of the isolates and were found in  
11 3–5 herds. Pairs of *S. aureus* isolates from persistent intramammary infections (IMI),  
12 repeated vaginal swabs, and from milk and teat skin from the same animal were usually  
13 identical. Paired isolates from other body sites of the same animal, including from bilateral  
14 IMI, were identical in less than 50% of the situations. The majority (71.9%) of analysed *S.*  
15 *aureus* isolates were *se*-positive. The genes *sec*, *sell* and *tst* were detected almost exclusively,  
16 but no correlation was observed between persistence of IMI and the enterotoxin gene profile  
17 of the causal *S. aureus* strains. The frequent presence of *S. aureus* on the mucous membranes  
18 may contribute to dispersal of the bacteria among dairy goats, hampering effective  
19 transmission control in dairy goat herds.

20 **Keywords:** *Staphylococcus aureus*; dairy goats; epidemiology; typing; enterotoxin genes

21

22

## 1 **1. Introduction**

2 *Staphylococcus aureus* is an important udder pathogen in small ruminants (Bergonier et al.,  
3 2003; White, 2007), and infected udders represent a source of bacterial spread and  
4 contamination of raw milk and raw milk products (Headrick et al., 1998; Jørgensen et al.,  
5 2005b; Zottola and Smith, 1993).

6 The infected mammary gland is often considered to be the primary reservoir of *S. aureus* in  
7 ruminants, but *S. aureus* may also be isolated from skin, especially from udder and teat skin,  
8 and from the mucous membranes of the nose and vagina of both cattle (Davidson, 1961;  
9 Haveri et al., 2008; Jørgensen et al., 2005b; Roberson et al., 1994) and small ruminants  
10 (Bergonier et al., 2003; Valle et al., 1991; Vautor et al., 2005; White, 2007). However, the  
11 importance of extramammary reservoirs of *S. aureus* remains to be elucidated, especially for  
12 small ruminants.

13 The virulence of *S. aureus* is believed, in part, to be conferred by exotoxins, including the  
14 staphylococcal enterotoxins (SE) and SE-like proteins (SEI) (Foster, 2005). Both the SEs and  
15 SEIs are superantigens (SAg) and the SEs cause emesis in primates when ingested (Dinges et  
16 al., 2000). The exact role of SAGs in staphylococcal infections remains unclear, but they may  
17 contribute to the pathogenesis of *S. aureus* intramammary infections (IMI) in ruminants by  
18 inducing aberrant activation of T cell populations which suppresses the immune response  
19 (Ferens et al., 1998; Park et al., 2006).

20 The main goal of this study was to identify reservoirs of *S. aureus* in dairy goats, and to  
21 compare isolates from milk, teat skin, and from the mucous membranes of the nose and  
22 vagina by pulsed-field gel electrophoresis (PFGE). The second goal was to investigate the  
23 prevalence of enterotoxin determinants among retrieved *S. aureus* isolates, and to determine  
24 whether or not there are differences in the SE-gene profiles of *S. aureus* isolates that cause  
25 persistent vs. those that cause temporary IMI in dairy goats.

1

## 2 **2. Material and Methods**

### 3 *2.1. Herds*

4 Seven commercial dairy goat herds (designated A–G) from 5 different counties in Norway  
5 were included in the study. They were selected following an advertisement asking for  
6 voluntary participants. The flock sizes varied from 35 to 280 milking goats. All the herds used  
7 machine milking, and the animals were housed during the winter, and grazed on mountain  
8 pastures during the summer. Small ruminant lentiviruses had been eradicated in herd A, C, D,  
9 E and F (<http://leine.no/htg/sanering/>). The newborn kids were, in all 7 herds, separated from  
10 their mothers at birth or within a few hours of birth.

11

### 12 *2.2. Samples*

13 Samples were collected between August 2005 and September 2006. Each farm was visited 4  
14 times in order to follow one lactation; before drying off, within 4 weeks of kidding, at the  
15 onset of the outdoor season, and again at drying off.

16 Milk samples from both udder halves from all lactating goats were collected at each farm  
17 visit, with the exception of herd A where all animals were sampled only at the first visit. From  
18 this herd, 100 lactating goats were randomly selected for sampling at the next 3 visits.

19 Body site swab samples from teat skin and the mucous membranes of the nose and vagina  
20 were collected from a random subset of the lactating goats in farms A–D within 4 weeks of  
21 kidding and at the time of drying off in 2006.

22 In addition, swab samples from teat skin and the mucous membranes of the nose and vagina  
23 were collected from a random selection of kids within 4 weeks after birth (n=16) and 6-8  
24 month after birth (n=23) in farms A–D.

25 Samples from animals with clinical mastitis were not included in the study.

1

2 *2.3. Sample collection*

3 All samples were collected by 7 veterinarians (one for each farm) following detailed  
4 instructions. They wore disposable latex gloves that were changed between each animal.

5 Udder secretions were collected aseptically in 10-ml sterile plastic vials as recommended by  
6 the International Dairy Federation (1981), frozen and kept at  $-20^{\circ}\text{C}$  until submission by  
7 express mail to the National Veterinary Institute (NVI) in Oslo.

8 Swab samples were collected using coal swabs (Eurotubo®, Rubi, Spain) moistened in sterile  
9 saline. Separate swabs were used for each sampling site. Teat skin samples were collected by  
10 moving a swab back and forth over the external surface of one teat. Nasal swabs were  
11 collected by inserting the same swab into both nostrils and gently rolling it against the nasal  
12 wall. The vaginal mucosa was sampled by inserting a swab 1–5 cm into the vagina and  
13 rotating it. All swabs were sent chilled in polystyrene boxes by express mail to the NVI,  
14 where microbiological analyses were started upon arrival.

15

16 *2.4. Bacteriological procedures and identification of S. aureus*

17 Microbiological analyses of udder secretions were performed essentially as recommended by  
18 the International Dairy Federation (1981). Briefly, milk samples were brought to room  
19 temperature, shaken, and 10  $\mu\text{l}$  were plated on blood agar (BA) (Oxoid, Basingstoke, United  
20 Kingdom) containing 5% washed bovine erythrocytes.

21 The swabs were placed in 10 ml Voegel-Johnson broth (Oxoid) with 0.5% agar in sterile glass  
22 test tubes, vortexed and incubated at  $37^{\circ}\text{C}$  for 4 h. One hundred microliters of the broth was  
23 then plated on Baird Parker agar with a Rabbit Plasma Fibrinogen supplement (BP+RPF;  
24 bioMérieux, Marcy-l'Etoile, France).

25 All agar plates were incubated at  $37^{\circ}\text{C}$  for 48 h and read after 24 and 48 h.

1 From BA, suspected staphylococcal colonies were Gram-stained, tested for catalase  
2 production and for coagulase by the tube coagulase test (Becton Dickinson, Sparks, Md).  
3 Gram-positive cocci, that were catalase- and coagulase-positive were streaked on peptone  
4 agar (p-agar) (Difco, Sparks, Md.) supplemented with 7 mg/l of acriflavin (Sigma-Aldrich  
5 Chemie, Steinheim, Germany). Typical colonies from BP+RPF (glistening black and  
6 surrounded by an opaque halo) were considered to be coagulase-positive staphylococci and  
7 were streaked directly onto p-agar with acriflavine. Some colonies without halos on BP+RPF  
8 were tested for coagulase production as described above prior to plating of p-agar. P-agar  
9 plates were incubated at 37°C for 24 h, and bacterial growth in the full length of the streak  
10 was considered to be a positive reaction and confirmation of *S. aureus* (Capurro et al., 1999).  
11 One or two selected colonies were purified and frozen in heart infusion broth (Difco) with  
12 15% glycerol at -70°C.

13

#### 14 2.5. Isolates for PFGE

15 Before PFGE-results were available, infections and colonizations were predefined as  
16 “persistent” when *S. aureus* was detected in 2 consecutive samplings and as “temporary”  
17 when a positive sample was followed by a negative sample. These definitions were used when  
18 selecting isolates for PFGE.

19 A total of 505 *S. aureus* isolates were selected for PFGE analyses.

20 To investigate whether bilateral IMI are caused by the same or different *S. aureus* genotypes 28  
21 pairs of isolates (n=56) from bilateral IMI were selected. Each pair included one isolate collected  
22 from each mammary gland of the same animal on the same day.

23 To investigate whether persistent IMI are caused by the same or different *S. aureus* genotypes 76  
24 pairs of isolates (n=152) from persistent IMI were selected. Each pair included one isolate  
25 collected from 2 consecutive samplings of the same mammary gland.



1 To investigate whether persistent extramammary colonizations are caused by the same or different  
2 *S. aureus* genotypes 40 pairs of isolates (n=80) collected from persistent extramammary  
3 colonizations were selected. Each pair included one isolate collected from 2 consecutive  
4 samplings of the same body site.

5 To investigate whether goats may carry several *S. aureus* genotypes at the same time 194 isolates  
6 were selected from 76 goats collected at 84 samplings. Isolates obtained from one goat collected  
7 on the same day were compared with each other.

8 Finally, 20 isolates from unbred kids and 53 isolates from temporary IMI were selected for PFGE.

9

## 10 2.6. PFGE

11 Preparation of chromosomal DNA and enzymatic digestion with *Sma*I were performed as  
12 described by Bannerman et al. (1995) with previously described modifications (Mørk et al.,  
13 2005).

14

## 15 2.7. Differentiation of PFGE profiles

16 Differentiation of PFGE banding patterns was performed using BioNumerics (version 4.0;  
17 Applied Maths, Kortrijk, Belgium) and by visual inspection. DNA fragments between  
18 approximately 45 kb and 650 kb were included in the analysis, and a unique electrophoretic  
19 banding pattern was defined as a pulsotype (PT).

20 One representative for each PT was selected to create a dendrogram using BioNumerics.

21 Pairwise similarity coefficients were calculated using the Dice formula, and a dendrogram  
22 was created using the unweighted pair group method with arithmetic averages. Optimization  
23 and position tolerance settings were 1% and 1.5%, respectively.

24

## 25 2.8. PCR for *se-* and *sel-*genes

1 In order to investigate whether or not potentially enterotoxigenic *S. aureus* isolates are more  
2 likely to cause persistent infections of the caprine mammary gland than *se*-negative isolates,  
3 153 PFGE-analyzed isolates from persistent (n=100) and temporary (n=53) IMI were selected  
4 for *se*- and *sel*-gene screening. All 153 isolates were tested for the genes *sea-see*, *seg-selj* by  
5 a multiplex PCR method (m-PCR) (Lovseth et al., 2004). In addition, 110 of the isolates were  
6 tested by conventional PCR for *selk-selo*, *selq*, *selr* and *selu*. Primers are listed in Table 1.  
7 These 110 isolates were selected as follows; all isolates from temporary IMI were included  
8 (n=53). If the 2 isolates in a pair from persistent IMI were identical by PFGE, one of the  
9 isolates was randomly selected (n=43), while if the 2 isolates of a pair were different by  
10 PFGE, both isolates were tested (n=14).

11 For DNA extraction, bacterial isolates were grown over night at 37°C with shaking in heart  
12 infusion broth (Difco). Cells were pelleted and DNA was extracted using NucliSens®  
13 easyMAG™ (bioMérieux).

14 The PCR program included denaturation at 95°C for 10 min followed by 35 cycles of 20 s  
15 denaturation at 95°C, 20 s annealing at 55°C, and 20 s extension at 72°C. A final extension  
16 for 10 min at 72°C completed the amplification. A set of in house *S. aureus* isolates that  
17 between them contained all the genes tested for in the PCR were included as positive controls  
18 for the PCR. Milli-Q water was used as negative control.

19

## 20 2.9. Statistical analyses

21 The difference between *S. aureus* isolates from persistent vs. temporary IMI with respect to  
22 *se*-gene profiles (*se*-positive or *se*-negative) were compared using contingency table analyses  
23 and the Chi squared statistic using JMP® version 7.0.1 (SAS institute Inc., Cary, NC, USA).  
24 Results were considered significant if  $p < 0.05$ .

25

### 1 3. Results

#### 2 3.1. Isolation of *S. aureus*

3 Results from the bacteriological analyses with respect to sampling sites and herds are  
4 presented in Table 2. *S. aureus* was detected in 353 (6.2%) of the 5671 milk samples and in  
5 625 (37.4%) of the 1673 swab samples from lactating goats. From kids, *S. aureus* was isolated  
6 from one (4%), 28 (61%) and 3 (8%) swab samples from teat skin, nose and vagina,  
7 respectively.

8 A noticeably higher percentage of vaginal swab samples were *S. aureus*-positive after kidding  
9 (44.9%) than before drying off (19.1%). Conversely, a higher percentage of nasal swab  
10 samples were *S. aureus*-positive before drying off (75.6%) than after kidding (62.0%).

11 Altogether 122 goats with *S. aureus*-positive nasal swabs after kidding were resampled before  
12 drying off and 90 (73.8%) of these were *S. aureus*-positive. Eighty two goats that had *S.*  
13 *aureus*-positive vaginal swabs after kidding were resampled before drying off and 22 (26.8%)  
14 were still *S. aureus*-positive.

15

#### 16 3.2. PFGE

17 All the 505 analysed *S. aureus* isolates were typeable by PFGE, and were divided into 33  
18 different PTs (Fig. 1). In a dendrogram with one representative for each PT, 6 different  
19 clusters (A–F) were defined visually. The minimum similarity between PTs within these  
20 clusters was 68.7% (Fig 1). Pairwise comparisons of the 33 PTs showed that with 4  
21 exceptions all PTs within a cluster differed by <6 bands and all PTs differed by >5 bands to  
22 all PTs outside its cluster.

23 The most prevalent PT (C2) comprised 116 (23.0%) isolates. Two PTs, C4 and C11, each  
24 differed by 2 and 4 bands from C2 and comprised 111 (22.0%) and 67 (13.3%) of the isolates,  
25 respectively. Three hundred and seventy (73.3%) isolates belonged to the 5 most prevalent

1 PTs (C2, C4, C11, A3, A1) and each of these PTs were found in 3–5 herds. With the  
2 exception of PT A1, isolates belonging to these 5 PTs were obtained from all the 4 major  
3 sampling sites. With 2 exceptions, C2, C4 and C11 were the predominant PT in each of the  
4 herds where they were found.

5 Within each herd, 4 to 15 different PTs were identified (Table 3). The greatest diversity was  
6 found in herd A with 15 PTs assigned to 4 clusters. PTs from more than one cluster were  
7 found in all herds except for herd E. Each of the 7 herds had a predominant PT which  
8 included 34 to 74% of the analysed isolates from that herd.

9 Comparisons of PTs of paired *S. aureus* isolates from bilateral IMI, persistent IMI, persistent  
10 body site colonizations and of isolates collected from IMI and extramammary sites of the  
11 same goat on the same day are shown in Table 4. The majority of isolate-pairs from persistent  
12 IMI, from repeated vaginal swabs and from milk and teat skin were identical, while remaining  
13 paired isolates were identical in less than 50% of the situations.

14 Out of the paired *S. aureus* isolates that had different PTs, the number of pairs that differed by  
15  $\leq 3$  and  $> 5$  band differences are presented in Table 4. Noticeably, in the majority of situations  
16 where paired *S. aureus* isolates from bilateral IMI, from milk and the nose, and from milk and  
17 the vagina were different, the PTs differed by  $> 5$  bands. In situations where paired isolates  
18 from persistent IMI, from milk and teat skin, from the nose (repeated samples), and from the  
19 vagina (repeated samples) had different PTs, most of the pairs differed by 3 bands or less.

20 When *S. aureus* isolates (n=194) from 76 goats, collected at 84 samplings were compared,  
21 different PTs were found among isolates from 55 (65.5%) of the samplings. From 36 (65.5%)  
22 of the samplings where isolates had different PTs,  $> 5$  band differences were found.

23

24 *3.3. Toxin gene detection*

1 Out of the 153 isolates tested by the m-PCR (for *sea-see*, *seg-selj* and *tst*), at least one SE-  
2 gene was detected in 110 (71.9%) of the isolates (Table 3). The additional testing of 110 of  
3 the isolates by conventional PCR (for *selk-selo*, *selq*, *selr* and *selu*) did not increase the number  
4 of *se*-positive isolates.

5 There was no significant difference between the *se*-profiles of isolates from predefined  
6 persistent vs. temporary IMI. This result remained the same also when the 7 pairs of isolates  
7 that were different by PFGE, but that had been predefined as from persistent IMI, were  
8 excluded from the analysis.

9 By m-PCR the genes *sec* and *tst* were found in 109 (71.2%) isolates and were always co-  
10 detected. Seventy eight of these isolates were also tested by conventional PCR and all were  
11 *sell*-positive. Three isolates from two animals were positive for *seg*, and *sei* by m-PCR. Two  
12 of these isolates, from the same animal, were also positive for *sec* and *tst*. Conventional PCR  
13 was performed on 2 of the *seg*- and *sei*-positive isolates (one from each animal), and both were  
14 positive for *selm*, *seln*, *selo* and *selu*. The genes *sea*, *seb*, *sed*, *selk*, *selq* and *selr* were not  
15 detected in any of the isolates.

16 The 153 isolates tested for SE-genes belonged to 22 of the PTs described above. There was a  
17 noticeable correspondence between SE-gene profile and PT and cluster (Fig. 1). With only 2  
18 exceptions, isolates belonging to cluster A (PTs A1–A5 and A7) were all *se*-negative.  
19 Conversely, most isolates (87.7%) belonging to cluster C were positive for *sec* and *tst* (and  
20 *sell*).

21

#### 22 4. Discussion

23 In the present study, 6.2% of the goat milk samples were *S. aureus* positive, with a variation  
24 at herd level between 2.4 and 11.4%. In earlier studies *S. aureus* has generally been isolated  
25 from less than 3% of the goats (Menzies and Ramanoon, 2001).

1 The nose was the extramammary body site most often colonized with *S. aureus*. Almost 70%  
2 of the sampled goats and 61% of the kids had *S. aureus*-positive nasal swabs. This indicates  
3 that the nose is an important colonization site for *S. aureus* in goats, and that kids are  
4 colonized during or soon after birth. Previous reports have indicated a *S. aureus* nasal carriage  
5 rate of 30% in dairy sheep (Vautor et al., 2005) and 6% in dairy goats (Valle et al., 1991), and  
6 that 9% of dairy heifers were colonized with *S. aureus* in the muzzle area (Roberson et al.,  
7 1994). On the other hand, in 2 studies from Norway (Jørgensen et al., 2005b) and the United  
8 Kingdom (Smith et al., 2005), respectively, *S. aureus* was not found in nasal swabs from dairy  
9 cows despite a high frequency of *S. aureus* IMI in both herds.

10 A higher *S. aureus* colonization rate was found in the vagina after kidding (45%) than before  
11 drying off (19%). Uterine involution and decreased secretions may explain the lower  
12 colonization rate before drying off. It is likely that presence of *S. aureus* on the mucous  
13 membranes of the vagina and in vaginal secretions contributes to the spread of *S. aureus* from  
14 goats to kids. The kids sampled in this study were removed from their mothers immediately  
15 after birth and received one feed of colostrum. They are unlikely, therefore, to have picked up  
16 *S. aureus* during muzzling and suckling.

17 Although the PFGE analyses revealed a great genetic diversity among the *S. aureus* isolates it  
18 was also evident that certain genotypes dominated. This was the case both when comparing  
19 isolates from individual herds, and when comparing the complete set of 505 isolates from all 7  
20 herds. The 3 most commonly observed PTs were, with 2 exceptions, also the dominant PT in  
21 the herds where they were found. It appears that *S. aureus* strains belonging to certain  
22 genotypes are more successful at spreading in the Norwegian dairy goat population than  
23 others. This is in agreement with a previous study where a limited number of closely related  
24 PTs were found responsible for a great proportion of the cases of *S. aureus* mastitis in dairy

1 cows, dairy goats and meat-producing sheep (Mørk et al., 2005). Similar findings have been  
2 reported regarding *S. aureus* in dairy sheep (Vautor et al., 2003).

3 In the present study almost all the included goats were colonized or infected with *S. aureus*,  
4 and individual goats could be colonised with several *S. aureus* genotypes. In fact in 65% of  
5 the situations, where more than one body site on the same goat was *S. aureus*-positive after  
6 sampling on the same day, isolates belonged to different PTs.

7 The majority of *S. aureus* IMI that were predefined as persistent were confirmed as persistent  
8 infections because two consecutively collected isolates were identical by PFGE. Isolating the  
9 same genotype on three consecutive occasions would have strengthened the definition of these  
10 infections as truly persistent, but this was not practicable in this study.

11 Although 18 out of the 76 compared *S. aureus* pairs had different PTs, 14 of these differed by  
12 only 2 bands. It could be argued that these belonged to the same infection because an infecting  
13 strain may undergo genetic change during the course of an infection (Tenover et al., 1995).

14 In contrast to the situation with persistent IMI, bilateral IMI were equally often caused by  
15 distinguishable PTs as by identical PTs. In the majority of situations where *S. aureus* pairs  
16 differed by PFGE, the PTs also belonged to different clusters. This indicates that *S. aureus*  
17 from different sources most often caused IMI in the 2 udder halves of bilateral IMI.

18 The nares were found to be a major *S. aureus* colonization site in Norwegian dairy goats. One  
19 might have expected that the nasal strain would also be the most frequent cause of IMI in  
20 individual goats. However, more than 50% of the isolate pairs, collected from the nose and an  
21 IMI of the same goat on the same day, differed by 4 bands or more by PFGE. A similar  
22 observation was made regarding pairs of isolates from the vagina and an IMI. This indicates  
23 that the mucous membranes are not the main reservoir of *S. aureus* IMI for individual goats.  
24 However, the frequent presence of *S. aureus* on the mucous membranes probably contributes  
25 to extensive dispersal of the bacteria in the environment, hampering effective transmission

1 control in the herds. Out of the paired isolates from repeated nasal swabs, that were compared  
2 by PFGE, 25% of the pairs differed by >5 band. This indicates frequent strain change in the  
3 nasal membranes consistent with intermittent rather than persistent carriage (Kluytmans et al.,  
4 1997).

5 In agreement with previous observations (Jørgensen et al., 2005a) and investigations by others  
6 (da Silva et al., 2005; Haveri et al., 2007; Scherrer et al., 2004; Smyth et al., 2005), the  
7 majority of analysed *S. aureus* isolates were SE-gene positive. The genes *sec*, *sell* and *tst* were  
8 detected almost exclusively, and indicates a wide distribution of the pathogenicity island  
9 Sap1bov (Fitzgerald et al., 2001b) among caprine *S. aureus* in Norway. It seems plausible that  
10 the toxins encoded by Sap1bov play a role in survival of *S. aureus* on its caprine host.  
11 However, no correlation was found between persistence of IMI and the SE-gene profile of the  
12 causal *S. aureus* strains. The observation that certain *S. aureus* PTs and PFGE clusters mostly  
13 had the same SE-gene profile indicates that it is clonal spread of toxin-encoding *S. aureus*  
14 strains rather than horizontal transfer of the toxin encoding genetic elements that is  
15 responsible for the wide distribution of Sap1bov.

16 In conclusion, *S. aureus* is frequently present on the mucous membranes of the nose and  
17 vagina of Norwegian dairy goats and the epidemiology is complex. The presence of *S. aureus*  
18 on other body sites than the mammary gland may contribute to the spread of *S. aureus* in dairy  
19 goat herds and thus add to mastitis control problems. The toxin genes *sec*, *sell* and *tst* were  
20 found to be widespread among the caprine *S. aureus* isolates, but the role of these toxins in  
21 colonizations and infections of goats remain to be elucidated.

22

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- 3

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- 1 **Table 1:** PCR primers used for detection of genes encoding staphylococcal enterotoxins
- 2 SEK–SEO, SEQ, SER and SEU
- 3

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1 **Table 2:** The number of different lactating goats sampled, the number of samples, and the  
2 frequency of *S. aureus* isolation from different sampling sites in 7 dairy goat herds  
3

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1 **Table 3:** Pulsed-field gel electrophoresis (PFGE) banding patterns (pulsotypes=PTs) and  
2 enterotoxin genes (SE-genes) among *S. aureus* isolates from 7 dairy goat herds  
3

Accepted Manuscript

1 **Table 4:** Comparisons of the pulsotypes (PTs) of paired *S. aureus* isolates from bilateral IMI;  
2 persistent IMI; persistent body site colonizations; and of isolates from IMI and an  
3 extramammary body site on the same goat on the same day

4

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1 **Figure 1:** Pulsed-field gel electrophoresis of *Sma*I digested DNA of *S. aureus* isolates  
2 representing the 33 pulsotypes (PTs) found in the 7 goat dairy herds. The name of the PTs, the  
3 presence in different farms, the total number of isolates analyzed by PFGE, the number of  
4 isolates analyzed by PCR and the enterotoxin gene profile (SE-gene profile) found are shown.

5 \* SE-neg = no SE-genes detected; SaP1bov = detected genes belonging to the  
6 pathogenicity island SaP1bov (*sec*, *sel* and *tst*) (Fitzgerald et al., 2001a); egc = detected genes  
7 belonging to the enterotoxin gene cluster (*egc*) (*sei*, *seg*, *selm*, *seln*, *selo*, and *selu*) (Jarraud et  
8 al., 2001).

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**Table 1:** PCR primers used for detection of genes encoding staphylococcal enterotoxins

SEK–SEO, SEQ, SER and SEU

Gene	Primer	Sequence 5'–3'	Product size	Accession number	Reference
<i>sek</i>	sek fwd	TTA GGT GTC TCT AAT AGT GCC AGC	278 bp		Smyth et al, 2005
	sek rev	AGC TGT GAC TCC GCC ATA TAT GTA			
<i>sel</i>	SEL-F158	CAC CAG AAT CAC ACC GCT TA	240 bp		Cremonesi et al., 2005
	SEL-R397	CTG TTT GAT GCT TGC CAT TG			
<i>sem</i>	mpSEM-1	CTA TTA ATC TTT GGG TTA ATG GAG AAC	300 bp		Jarraud et al., 2001
	mpSEM-2	TTC AGT TTC GAC AGT TTT GTT GTC AT			
<i>sen</i>	sen fwd	GCT TAT GAG ATT GTT CTA CAT AGC TGC	448 bp		Smyth et al, 2005
	sen rev	CAT TAA CGC CTA TAA CTT TCT CTT CAT C			
<i>seo</i>	seo fwd	AAG AAG TCA AGT GTA GAC CCT ATT GCT	201 bp	AF285760	*
	seo rev	AAT CGC TGA TGA GCT AAA TTC CAC			
<i>sep</i>	sep fwd	ATT TAC AAA AAA AGT CTG AAT TGC AGG	201 bp		Kuroda et al., 2001
	sep rev	TGG CGG TGT CTT TTG AAC C			
<i>seq</i>	seq fwd	TGG AAA ATA CAC TTT ATA TTC ACA GTT TCA	201 bp	AF410775.	*
	seq rev	TTT TGC TTA CCA TTG ACC CAG AG			
<i>ser</i>	ser fwd	CGG TTA GAT GTG TTT GGA ATA CCC	201 bp	AB075606	*
	ser rev	ATT TGT ACT AAT TGT AAA AGA GAA CTG TTG TTT			
<i>seu</i>	seu fwd	AAA ATA TGG AGT TGT TGG AAT GAA GTT	201 bp		Letertre et al., 2003
	seu rev	TTC TCT TGG GCT TTA ATG TTT GTT T			

\* designed in the present study.

**Table 2:** The number of different lactating goats sampled, the number of samples, and the frequency of *S. aureus* isolation from different sampling sites in 7 dairy goat herds

Milk samples, teat skin swabs, nasal swabs and vaginal swabs	Herd							All herds
	A	B	C	D	E	F	G	
<b>Milk</b>								
No. of goats sampled	327	138	111	92	138	39	176	1039
No. of samples	1239	916	702	701	920	245	948	5671
No. of <i>S. aureus</i> (%)	130 (10.5)	27 (2.9)	17 (2.4)	41 (5.8)	44 (4.8)	40 (11.4)	54 (5.7)	353 (6.2)
<b>Teat skin</b>								
No. of goats sampled	105	125	49	81				360
No. of samples	122	194	91	128				535
No. of <i>S. aureus</i> (%)	5 (4.1)	14 (7.2)	16 (17.6)	18 (14.1)				53 (9.9)
<b>Nasal membrane</b>								
No. of goats sampled	119	125	48	83				375
No. of goats sampled twice	27	68	43	56				194
No. of samples	146	193	91	139				569
No. of <i>S. aureus</i> (%)	102 (69.9)	118 (61.1)	68 (74.7)	104 (74.8)				392 (68.9)
<b>Vaginal membrane</b>								
No. of goats sampled	119	125	48	83				375
No. of goats sampled twice	27	68	43	56				194
No. of samples	146	193	91	139				569
No. of <i>S. aureus</i> (%)	47 (32.2)	60 (31.1)	28 (30.8)	45 (32.4)				180 (31.6)

**Table 3:** Pulsed-field gel electrophoresis (PFGE) banding patterns (pulsotypes=PTs) and enterotoxin genes (SE-genes) among *S. aureus* isolates from 7 dairy goat herds

	Herd							All herds
	A	B	C	D	E	F	G	
<b>PFGE<sup>a)</sup></b>								
No. of <i>S. aureus</i> analyzed	156	94	60	89	45	31	30	505
No. of PTs found	15	9	8	6	6	4	8	
<b>SE-genes</b>								
No. of <i>S. aureus</i> analyzed	34	16	7	14	23	29	30	153
No. of <i>se</i> -positive (%)	15 (44.1)	13 (81.2)	5 (71.4)	5 (35.7)	23 (100)	21 (72.4)	28 (93.3)	110 (71.9)

a) 5, 5, 7 and 3 isolates from unbred kids included from herd A, B, C and D, respectively.

**Table 4:** Comparisons of the pulsotypes (PTs) of paired *S. aureus* isolates from bilateral IMI; persistent IMI; persistent body site colonizations; and of isolates from IMI and an extramammary body site on the same goat on the same day

Sample sites	No. of pairs of <i>S. aureus</i>	No. of pairs with identical PTs (%)	No. of pairs with $\leq 3$ band differences out of pairs with different PTs (%)	No. of pairs with $>5$ band differences out of pairs with different PTs (%)
Bilateral IMI (milk)*	28	12 (42.9)	7 (43,8)	9 (56.2)
Persistent IMI (milk)	76	58 (76.3)	15 (83,3)	3 (16.7)
Milk-teat skin*	6	4 (75.0)	0 (0)	2 (100.0)
Milk-nasal membranes*	27	9 (33.3)	3 (16.7)	15 (83.3)
Milk-vaginal membranes*	12	3 (25.0)	1 (11.1)	7 (77.8)
Nasal membranes-repeated samples	36	15 (41.7)	11 (52.4)	9 (42.9)
Vaginal membranes-repeated samples	4	3 (75.0)	1 (100.0)	0 (0.0)

\*) isolates were collected from the same goat on the same sampling day.

Dice (CpI 1.00%) (Tol 1.5%-1.5%) (H=0.0% S=0.0%) (0.0%-100.0%)  
 PFGE PFGE

