



Differences between *Staphylococcus aureus* lineages isolated from ovine and caprine mastitis but not between isolates from clinical or subclinical mastitis

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ABSTRACT

Staphylococcus aureus is an important mastitis pathogen, causing both clinical mastitis (CM) and subclinical mastitis (SCM) in small ruminants. In general, CM has a low incidence in sheep and goats but can be very severe and costly. In contrast, subclinical mastitis (SCM) is common but is associated with less cost. For both sheep and goats, *S. aureus* is the main cause of CM and is associated with SCM cases with a high SCC. Recently, specific lineages of *S. aureus* have been identified that are associated with CM rather than SCM in dairy cows. It is unknown whether specific *S. aureus* lineages are associated with CM in goats and sheep. The aim of this study was to compare the clonal complex (CC), staphylococcal protein A (*spa*) type, leukocidin *lukM-lukF*⁷ presence, and potential to produce LukMF⁷ in vitro between CM and SCM *S. aureus* mastitis isolates obtained from sheep and goats. Differences between isolates from different host species were also compared. Ovine (CM, n = 12; SCM, n = 29) and caprine (CM, n = 14; SCM, n = 30) isolates were obtained from 8 sheep flocks and 8 goat herds in the Netherlands. Overall, the isolates belonged to CC133 (85%), CC398 (7%), CC425 (5%), and CC45 (2%). Seventeen *spa* types were found, including 6 novel types; the predominant types were t2678 (34%), t544 (18%), and t3583 (18%). Although CC133 was dominant among both sheep and goat isolates, *spa* type CC133/t2678 was associated with ovine isolates, whereas CC133/t544 and CC133/t3583 were found mostly in goats. The presence of *lukM-lukF*⁷ among the *S. aureus* isolates was high (87%), especially in CC133 (96%) and CC425 (100%), but the genes were absent in CC45 and CC398. In vitro-cultured *lukM-*

*lukF*⁷-positive isolates produced LukM (71 out of 74 positive isolates tested) in the range of 0.4 to 5.0 µg/mL. Interestingly, the goat-associated lineages CC133/t544 and CC133/t3583 produced more LukM in vitro than the sheep-associated CC133/t2678. We found no difference in LukMF⁷ production potential between CM and SCM isolates. In sheep as well as in goats, no association was found between genotype and CM or SCM, demonstrating that the same lineages of *S. aureus* are responsible for both CM and SCM. These results suggest that subclinically infected animals in a herd or flock likely act as the reservoir of *S. aureus* causing CM. This highlights the importance of early identification and control of SCM and suggests that controlling SCM within a herd is an effective intervention to prevent CM in small ruminants.

Key words: *Staphylococcus aureus*, mastitis, LukMF⁷, sheep, dairy goat

INTRODUCTION

Mastitis is a common disease among dairy goats and sheep that is responsible for economic losses due to reduced milk production (Gonzalo et al., 2002; Koop, 2012), decreased milk quality (Barrón-Bravo et al., 2013; Martí De Olives et al., 2013), increased lamb mortality (Arsenault et al., 2008), costs of treatment, and culling (Conington et al., 2008; Koop, 2012). Furthermore, behavioral differences are observed in animals suffering from mastitis; this, together with clinical symptoms, indicates a reduction in animal welfare (Gougoulis and Kyriazakis, 2010). Clinical mastitis (CM) is characterized by the classical signs of inflammation (pain, swelling, heat, erythema, and loss of function), causing visible abnormalities in milk or the udder (Smith and Sherman, 2009). In the present study, subclinical mastitis (SCM) is defined by a positive bacteriological culture from normal-looking milk in the absence

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of clinical symptoms (Koop et al., 2010). Because the use of elevated SCC as an indicator for SCM in small ruminants is debated (de los Campos et al., 2006; Koop et al., 2010), we chose not to consider it to define SCM. Although SCM is common in goats and sheep (Moroni et al., 2005; Vasileiou et al., 2018), it only results in a limited decrease in milk yield (Gonzalo et al., 2002; Koop et al., 2010; Martí De Olives et al., 2013) and relatively low costs for the farmer (Koop, 2012). Many SCM cases are caused by CNS but also by *Staphylococcus aureus* (Fthenakis, 1994; Moroni et al., 2005). In contrast, CM has a low incidence but is responsible for more economic losses than SCM (e.g., higher reduction in milk yield and quality, increased lamb mortality and the culling of animals), highlighting the importance of CM control to reduce the costs of mastitis on farms (Bergonier et al., 2003; Arsenaault et al., 2008; Koop, 2012; Martí De Olives et al., 2013). Most CM cases in sheep and goats are caused by *S. aureus* (Bergonier et al., 2003), and the dominant lineages of *S. aureus* isolated from these animals are clonal complex (CC) 133, CC130, and CC522 (Smith et al., 2014; Merz et al., 2016). Although several studies consider *S. aureus* isolates from goats and sheep to be part of the same population based on CC, typing techniques with higher resolution, such as staphylococcal protein A (*spa*) typing, do detect different *S. aureus* lineages associated with the 2 species (Porrero et al., 2012; Eriksson et al., 2013; Azara et al., 2017). Among CC133 isolates obtained from small ruminants, certain *spa* types were predominantly found in goats (t1166, t7304), and other types (t2678, t9088) were found in sheep (Porrero et al., 2012; Eriksson et al., 2013).

The severity of infectious diseases is determined by the pathogen, the host, and their interaction, but it is largely unclear to what extent the clinical severity of mastitis is driven by host or pathogen factors (Fournier et al., 2008; Rainard et al., 2018a). Recently, we identified a bovine-associated lineage of *S. aureus* (sequence-type 479) that was associated with CM rather than SCM in cattle (Hoekstra et al., 2018). High production of leukocidin LukMF', a ruminant-associated virulence factor that is a potent killer of ruminant neutrophils in vitro (Vrieling et al., 2016), by ST479 *S. aureus* was the likely explanation for this association with CM. To our knowledge, associations between *S. aureus* lineages and CM or SCM have not previously been investigated in sheep and goats.

The aim of this study was to describe the genetic diversity of *S. aureus* isolates obtained from cases of ovine and caprine mastitis in the Netherlands and to determine to what extent this variation is associated with CM or SCM and with host species. Multilocus sequence typing (MLST) and *spa* genotyping were performed,

and the proportion of isolates carrying *lukM-lukF'* and their LukMF' production potential were determined.

MATERIALS AND METHODS

Sample Collection

A convenience sample of 18 Dutch dairy goat farmers was asked to aseptically collect milk samples from any goat suffering from CM. For the present study, the definition of CM was visible abnormalities in the udder or milk or both. For every case of CM, farmers were asked to fill out a form to record goat ID, date of CM, date of kidding, parity, affected udder half, type of mastitis (gangrenous mastitis or nongangrenous mastitis), clinical symptoms, appearance of the milk, and treatment. The milk samples from CM were frozen at -20°C by the farmer. At the end of the sampling period, the samples were shipped to the Veterinary Microbiological Diagnostic Center at Utrecht University for bacteriological culture and speciated according to National Mastitis Council guidelines (Hogan, 1999), resulting in 14 *S. aureus* CM isolates from 8 herds. The SCM isolates ($n = 30$), originating from 5 herds that also contributed caprine CM isolates, were used from a previous study on SCM in dairy goats (Koop et al., 2010).

Ovine mastitis isolates were obtained from milk samples from 238 meat sheep from 15 herds. During sampling, udder halves showing abnormalities in appearance of the milk or the udder or both were classified as having CM. All milk samples were cultured on blood agar plates according to National Mastitis Council guidelines (Hogan, 1999), and bacterial species were determined using MALDI-TOF (Jang and Kim, 2018). A positive bacterial culture result in the absence of reported abnormalities in milk or the udder was classified as SCM. A total of 41 *S. aureus* isolates were found, originating from 8 herds, including 12 samples originating from cases classified as CM and 29 classified as SCM.

Genotyping of Isolates

Extraction of DNA from *S. aureus* isolates was performed using a boiling protocol as described by Hoekstra et al. (2018). The polymorphic X-region of the *spa* genes of the isolates was amplified according to the Ridom StaphType standard protocol (www.ridom.org). When standard *spa*-typing primers failed to yield a PCR product in an isolate, an alternative set of *spa* primers (Hallin et al., 2009) was used. Amplicons were purified using ExoSAP-IT PCR Cleanup Reagent (Affymetrix, Santa Clara, CA) following the manufacturer's instructions and sequenced using Sanger se-

quencing (Baseclear, Leiden, the Netherlands). Based on sequenced PCR amplicons, *spa* types were assigned using BioNumerics version 7.5 (Applied Maths, Sint-Martens-Latem, Belgium) and the *spa*-typing plugin. A minimum spanning tree of *spa* types was created in BioNumerics version 7.5 by the *spa* clustering methods of the *spa*-typing plugin using the same settings as described by Mekonnen et al. (2018).

For MLST, a subset of ovine isolates ($n = 10$) was selected, representing the diversity of *spa* types among ovine isolates. Typing was performed according to the protocol described on the MLST website (<http://saureus.mlst.net>). The MLST of caprine isolates was obtained from whole-genome sequence (WGS) data. For WGS, DNA was extracted using a MasterPure gram-positive DNA purification kit (Cambio, Cambridge, UK), and HiSeq sequencing was performed according to the manufacturer's protocol (Illumina Inc., San Diego, CA); genotyping was done based on the assembled genomes of these isolates.

Detection of *lukM-lukF'* and In Vitro LukM Production

The PCR amplification of *femA*, *lukM*, and *lukF'* and measurement of in vitro LukMF' production potential was performed as previously described by Hoekstra et al. (2018). Briefly, to measure the LukMF' production potential, isolates were grown in broth under controlled conditions for 8 h and the LukM concentration in broth was measured using ELISA (Vrieling et al., 2016; Hoekstra et al., 2018).

Presence of *lukM-lukF'* in WGS of *S. aureus* isolates was investigated by BLASTN using reference sequences for *lukM* (GenBank accession no. 1262967) and *lukF'* (GenBank accession no. 1262954). Identity scores higher than 95% were considered positive for *lukM* or *lukF'*.

Statistical Analysis

For the LukM ELISA, LukM values were extrapolated from a LukM standard curve using GraphPad Prism 7 software (GraphPad Software, La Jolla, CA). Differences in LukMF' production potential between different lineages were tested by Kruskal-Wallis test followed by Dunn's test in GraphPad Prism 7. To compensate for a possible farm effect on LukMF' production potential, a subset of the LukMF' production potential data set with only a single *spa*-type value per farm was also used. In case of multiple isolates with the same *spa* type on a farm, the averaged LukM production of isolates belonging to this *spa* type was used.

The Mann-Whitney test was used to compare differences in LukMF' production potential between *S. aureus* isolated from sheep and goats and from CM

and SCM (for all isolates and within ovine and caprine isolates). Differences in LukMF' production potential between *spa* types was only tested among types with $n > 10$ using the Kruskal-Wallis test followed by Dunn's test. Fisher's exact tests were performed to study the association between clinical severity and CC, *spa* type, and *lukM-lukF'*. Using the same technique, the association between host species and CC and *spa* type was also investigated. All tests were performed using IBM SPSS Statistics version 24 (IBM Corp., Armonk, NY).

RESULTS

The results of MLST, *spa* typing, and presence of *lukM-lukF'* among *S. aureus* isolates are reported in Table 1, and an overview of the distribution of CM and SCM over different *spa* types is shown in Figure 1. Most isolates (85%) obtained from goats and sheep belonged to CC133, and this lineage was present on 15 out of 16 farms (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16196>). Significant differences (Fisher's exact test, $P = 0.001$) were observed between the CC of caprine and ovine mastitis isolates, with CC398 being exclusively found in goats and CC425 and CC45 found only in sheep (Figure 1). Multiple *spa* types were present among CC133 isolates, the dominant types being t2678 (40% of CC133 isolates), t544 (21%), and t3583 (21%). The distribution of the dominant CC133 *spa* types differed between hosts (Fisher's exact test, $P < 0.001$). Isolates belonging to CC133/t544 (ovine: 0%, caprine: 34%; $P < 0.001$) and CC133/t3583 (ovine: 5%, caprine: 30%; $P = 0.004$) were associated with caprine mastitis, and those designated CC133/t2678 (ovine: 56%, caprine: 14%; $P < 0.001$) were associated with ovine mastitis (Table 1). The *spa* types t544, t3583, and t2678 were genetically related, as illustrated by the fact that they cluster together in the minimum spanning tree (Figure 1).

Presence of *lukM-lukF'* was common in CC133 and CC425 but absent in CC45 and CC398 (Table 1). The LukMF' production potential was measured in 71 *lukM-lukF'*-positive isolates, with production levels after 8 h of culture ranging from 0.4 to 5.0 $\mu\text{g}/\text{mL}$. The CM and SCM isolates produced similar levels of LukM both in ovine (CM: $2.0 \pm 0.9 \mu\text{g}/\text{mL}$, SCM: $2.0 \pm 0.8 \mu\text{g}/\text{mL}$; Mann-Whitney test: $P = 0.95$) and caprine (CM: $3.6 \pm 0.9 \mu\text{g}/\text{mL}$, SCM: $3.0 \pm 1.0 \mu\text{g}/\text{mL}$; Mann-Whitney test: $P = 0.07$) isolates separately and when comparing all isolates (CM: $2.8 \pm 1.2 \mu\text{g}/\text{mL}$, SCM: $2.5 \pm 1.0 \mu\text{g}/\text{mL}$; Mann-Whitney test: $P = 0.28$). Among the isolates belonging to the 3 dominant *spa* types (representing 76% of the total isolate collection), the ovine-associated CC133/t2678 isolates produced LukM at significantly lower levels ($1.9 \pm 0.8 \mu\text{g}/\text{mL}$) than the dominant caprine

Table 1. Clonal complex (CC), *spa* type, and *lukM-lukF*² carriage of 85 *Staphylococcus aureus* isolates obtained from cases of ovine and caprine mastitis from 8 goat herds and 8 sheep flocks in the Netherlands

CC	No. (%)	<i>spa</i> type	<i>spa</i> repeats ¹	Ovine isolates			Caprine isolates		
				No. (%)	<i>lukM-lukF</i> ² no. (%)	Clinical no. (%)	No. (%)	<i>lukM-lukF</i> ² no. (%)	Clinical no. (%)
133	73 (85)	t2678 t3583 t544 t16713 ³ t16712 ³ t16710 ³ t12382 t3495 t4560 t17048 ³ t17047 ³ NA ⁴	03-16-12-21-17-23-13-17-17-17-23-24 03-16-21-17-23-13-17-17-17-23-24 03-16-12-21-17-23-13-17-17-17-23-24 03-16-12-21 03-12-12-21-17-23-13-17-17-17-23-24 03-16-12-21-22-17-23-13-17-17-23-24 03-21-17-23-13-17-17-17-23-24 03-16-12-21-17-23-13-17-17-23-24 03-16-12-21-17-23-13-17-17-17-23-24 03-16-12-21-23-13-17-17-17-23-24 03-16-12-21-17-17-17-23-24	23 (56) 2 (5) 4 (8) 2 (5) 1 (2) 1 (2)	23 (100) 2 (100) 4 (100) 2 (100) 1 (100) 0 (0)	8 (35) 2 (100) 0 (0) 0 (0) 0 (0) 0 (0)	6 (14) 13 (30) 15 (34)	6 (100) 13 (100) 13 (87)	0 (0) 5 (38) 5 (33)
398	6 (7)	t011	NA	2 (5)	2 (100)	0 (0)	1 (2)	1 (100)	0 (0)
425	4 (5)	t15002 t16711 ³	08-16-02-25-34-24-25 14-44-12-12-17-23-18-17-17-17-23-24	2 (5) 2 (5)	2 (100) 2 (100)	0 (0) 1 (50)	6 (14)	0 (0)	3 (50)
45	2 (2)	t015 t715	14-44-12-12-17-23-18-17-17-17-23-24 08-16-02-16-34-13-17-34-16-34 09-02-16-34-13-17-34	1 (3) 1 (3)	0 (0) 0 (0)	0 (0) 1 (100)	1 (2)	1 (100)	0 (0) 1 (100)
Total	85 (100)			41 (100)	38 (93)	12 (29)	44 (100)	36 (82)	14 (32)

¹Numerical code of *spa* repeats chosen by Ridom StaphType (www.ridom.org).

²Number and percentage of *lukM-lukF*²-positive isolates.

³Novel *spa* types.

⁴Isolates not typeable using any of the *spa* primer sets used in our study.

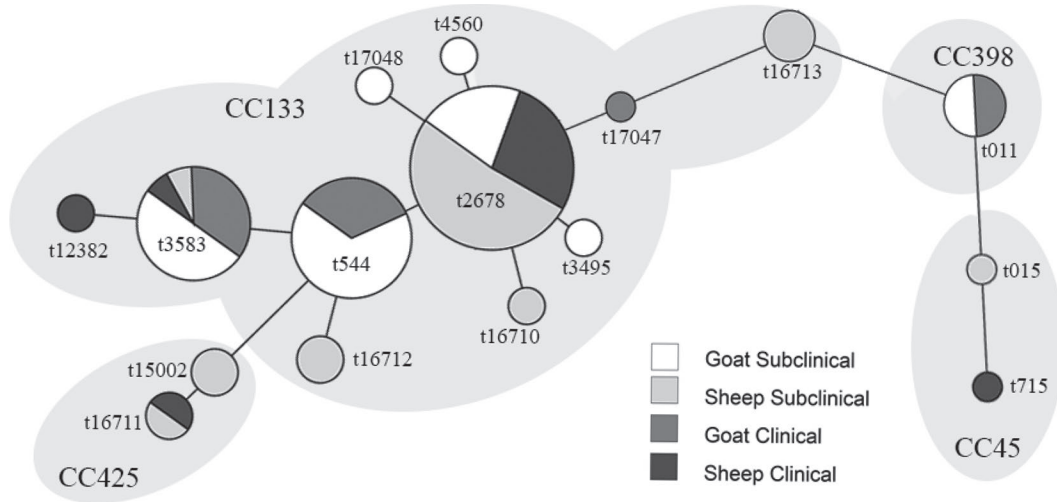


Figure 1. Minimum spanning tree of *spa* types of ovine ($n = 41$) and caprine ($n = 44$) *Staphylococcus aureus* mastitis isolates cultured from 16 Dutch farms. Each *spa* type is depicted by a single node, with the size of the node representing the number of isolates associated with this *spa* type and colors representing host species (goat, sheep) and clinical or subclinical mastitis. Associated clonal complex (CC) is displayed in shading.

rine CC133/t3583 ($3.5 \pm 0.7 \mu\text{g/mL}$, Dunn's test, $P < 0.001$) and CC133/t544 ($3.0 \pm 1.3 \mu\text{g/mL}$, Dunn's test, $P = 0.007$) isolates (Figure 2). A similar but not significant trend was seen in a subset of data using an averaged LukM value per *spa* type per farm (Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-16196>). Fisher's exact tests on all isolates revealed no association between SCM or CM and CC ($P = 0.52$), *spa* type ($P = 0.65$), or presence of *lukM-lukF'* ($P = 0.25$). Similar results were seen when the analysis was performed for ovine and caprine isolates separately (results not shown).

DISCUSSION

In this study, we compared the genotype and LukMF' production potential of *S. aureus* isolates originating from CM and SCM in both goats and sheep. Although most of the caprine and ovine isolates belonged to CC133, the dominant *spa* type within CC133 found in sheep differed from the ones in goats. Interestingly, isolates from these caprine-associated *spa* types produced higher levels of LukM in vitro than isolates from the sheep-associated *spa* type.

The majority of small ruminant mastitis *S. aureus* isolates in this study belonged to CC133, a lineage with a broad host range (Bar-Gal et al., 2015; Monecke et al., 2016), although it has primarily been associated with ruminants, in particular goats and sheep (Smith et al., 2014; Merz et al., 2016; Monecke et al., 2016). Similar observations were made in Denmark, where CC133 was also the dominant lineage found in sheep and goats

(Eriksson et al., 2013). Besides CC133, CC522 and CC130 lineages are considered to be dominant lineages associated with small ruminants (Smith et al., 2014),

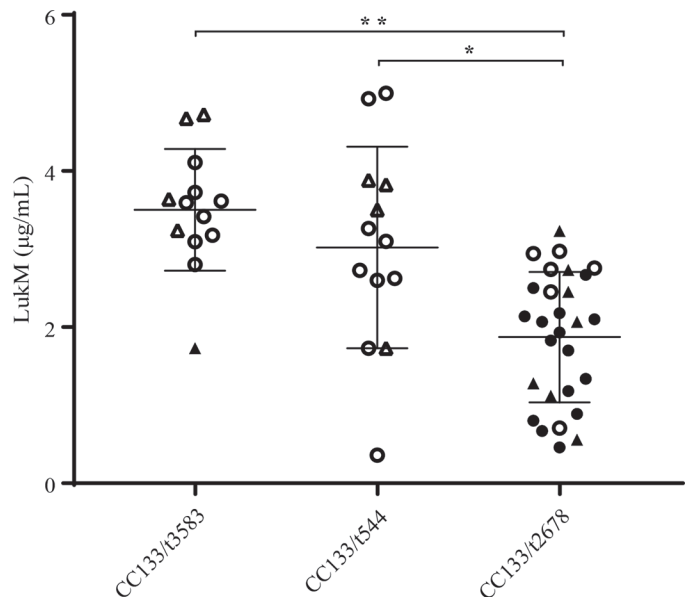


Figure 2. The LukM levels in vitro after 8 h of culture of *lukM-lukF'*-positive *Staphylococcus aureus* clinical mastitis (triangles) and subclinical mastitis (circles) isolates obtained from sheep (black) and goats (white) belonging to the 3 dominant genotypes, CC133/t3583 ($n = 13$), CC133/t544 ($n = 13$), and CC133/t2678 ($n = 28$), tested by ELISA. CC = clonal complex. Mean LukM production \pm SD are shown. Production potential of isolates ($n = 17$) belonging to 10 other *spa* types was measured but is not displayed in this figure. Statistical significance was analyzed by Kruskal-Wallis test followed by Dunn's test. Asterisks indicate a significant difference: ** $P < 0.01$, * $P < 0.05$.

but these types were not found in our study. Although *S. aureus* lineages found among sheep and goats are considered to be highly similar (Merz et al., 2016), systematic differences in *spa* types between isolates from the 2 host species were observed in this study, with CC133/t2678 being associated with ovine mastitis and CC133/t3583 and CC133/t544 being associated with caprine mastitis. In addition, there were differences in LukMF' production potential, with CC133/t544 and CC133/t3583 producing on average around 1.5 times more LukM than CC133/t2678. A possible explanation for variation in LukMF' production potential is differences in expression levels of genes involved in the regulation of leukocidin production (Agr quorum-sensing system, SaeRS 2-component systems, Rot; Alonzo and Torres, 2014). Differences in *spa* repeats between the 3 dominant *spa* types were small, and CC133/t2678 and CC133/t3583 were not exclusively associated with one host species. Furthermore, previous studies have also described CC133/t544 isolates in sheep (Porrero et al., 2012; Eriksson et al., 2013). Differences in *spa* type between CC133 *S. aureus* obtained from sheep and goats have been observed in multiple studies, but the dominant *spa* type per host species differed per country (Porrero et al., 2012; Eriksson et al., 2013; Azara et al., 2017). It is unclear whether differences in *spa* type between sheep and goats reflect functional adaptations of the *S. aureus* lineage to the host.

Lineages other than CC133 (CC398, CC425, and CC45) made up only 14% of mastitis isolates in this study, and all of these lineages have previously been isolated from goats and sheep (Bergonier et al., 2014; Cortimiglia et al., 2015). We found the CC398/t011 lineage only in goats, and CC398 is the predominant lineage of livestock-associated methicillin-resistant *S. aureus* in Europe (Richardson et al., 2018). The CC425 lineage is predominantly associated with *S. aureus* from wildlife origins (Monecke et al., 2016) and was found in only a single sheep flock.

The virulence genes *lukM-lukF'* were present among most isolates, and we found no relationship between presence of *lukM-lukF'* or LukMF' production potential and clinical severity of mastitis in sheep or goats. Previously, *lukM-lukF'*-positive *S. aureus* was associated with CM in cattle (Haveri et al., 2007), and we found that high production of LukMF' was linked with bovine CM (Hoekstra et al., 2018). Presence of *lukM-lukF'* and production potential of LukMF' are associated with CC (Schlotter et al., 2012; Hoekstra et al., 2018). Most of our isolates (85%) belong to a single CC, and this could explain why we found no differences in presence and production potential of LukMF' between CM and SCM isolates.

The association between *lukM-lukF'* and CC133 has previously been reported in bovine, ovine, and caprine isolates (Schlotter et al., 2012; Bar-Gal et al., 2015). However, 2 CC133/t544 isolates, obtained from the same goat herd, lacked *lukM-lukF'*. Genome analysis revealed that the prophage associated with *lukM-lukF'* (phiPV-83; Yamada et al., 2005) was still present in these isolates but lacked the region containing the actual leukocidin genes. All CC425 and the majority of CC133 isolates found in our study carried *lukM-lukF'*, whereas CC425 and CC133 obtained from wildlife ruminant hosts (red deer, roe deer, chamois) rarely harbored *lukM-lukF'* (Monecke et al., 2016). This could suggest that harboring *lukM-lukF'* gives *S. aureus* increased fitness within domesticated ruminants but not within wildlife species.

We found no differences between CM and SCM isolates in sheep or goats. This suggests that the clinical manifestation of mastitis is driven by host factors rather than by the pathogen *S. aureus*. Still, it is possible that differences between *S. aureus* isolates beyond the resolution of the typing methods used in our study determine the outcome of IMI. However, a recent infection study in goats using 2 lineages of *S. aureus* showed that host factors determined the clinical manifestation of mastitis because animals infected with the same *S. aureus* strain had different clinical outcomes (Rainard et al., 2018b).

Mastitis isolates were collected from 8 sheep flocks and 8 goat herds across the Netherlands, but there was an uneven distribution of the number of isolates per farm (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16196>). As a consequence, farm is a potential confounder for observed differences in LukMF' production potential. To compensate for this, a subset using the averaged LukM value per *spa* type per farm was formed, and similar trends in differences in LukMF' production potential were still present in this subset. The uneven distribution of isolates can also result in over- or underrepresentation of *S. aureus* lineages in our data set, especially in the caprine isolates, because one farm contributed a large number (43%) of the caprine isolates (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16196>). However, the *spa* types found within that goat farm were similar to *spa* types found on other farms, and the overall variation in *spa* types between caprine isolates was low. Therefore, we do not expect that this sampling bias substantially affected our main finding that there was no association between genotype and clinical outcome of infection.

Because persistent SCM cases can develop into CM at a later point in time (Lam, 1996; Zadoks et al., 2002), the same strain may be associated with both CM

and SCM depending on the time of sampling. Additionally, the clinical outcome of an infection is at least in part determined by host factors (Rainard et al., 2018b). This illustrates that the CM or SCM phenotype classification of an isolate based on a single mastitis case is imperfect. However, other studies using the same classification did identify strain variation in pathogenicity of bovine mastitis (Haveri et al., 2007; Hoekstra et al., 2018; Pichette-Jollete et al., 2019). This demonstrates that despite misclassification of strains and the resulting reduction in power, our study approach should in principle be able to identify pathogen-related factors that contribute to severity of an intramammary *S. aureus* infection.

Although most mastitis isolates obtained from both sheep and goats belonged to CC133, the CC133/t2678 lineage was associated with ovine mastitis and CC133/t544 and CC133/t3583 were associated with caprine mastitis. We found no significant differences between *S. aureus* isolated from CM or SCM cases originating from small ruminants, implying that animals with SCM are a reservoir of *S. aureus* responsible for CM. This finding suggests that controlling SCM within a herd is an effective intervention to prevent CM in small ruminants.

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