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Persistence of coagulase negative staphylococcal intramammary infections in dairy goats

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Research Article

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Abstract

The objectives of the research described here were to describe the persistence of intramammary infections (IMI) caused by coagulase negative staphylococci (CNS) in goats using strain-typing, and to evaluate the relationship between species-specific CNS IMI and somatic cell score (SCS) at the udder-half level. Udder-half milk samples were collected from all 909 lactating goats (1817 halves; 1 blind half) in a single herd. Milk samples were cultured on Columbia blood agar, and 220 goats with at least one half yielding a single colony type CNS were enrolled for two additional half-level samplings at approximately 1-month intervals. Isolates were identified to the species level by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry or PCR amplification and partial sequencing of *tuf* or *rpoB*. An IMI was defined as persistent when ≥1 follow-up sample yielded the same species and strain as on Day 0 based on pulsed-field gel electrophoresis. A generalised mixed linear model was used to evaluate the odds of persistence as a function of CNS species. A mixed linear model was used to evaluate the relationship between IMI status on a given day and SCS. Among 192 IMI, 69.8% were persistent based on species and strain-type. *Staphylococcus simulans* IMI had higher odds of persistence than *Staphylococcus arlettae* IMI. In primiparous goats, *Staphylococcus epidermidis* IMI was associated with higher SCS than *S. arlettae*, *Staphylococcus xylosus* and 'other CNS' IMI. The differences detected in the present study between CNS species, with regard to persistence of IMI and association with SCS, highlight the need to study CNS at the species and strain level to understand the pathogenicity and epidemiology of CNS in goats.

Staphylococci are the most common bacteria causing subclinical intramammary infection (IMI) in dairy goats, with a higher prevalence of coagulase-negative staphylococci (CNS) than of *Staphylococcus aureus* (Bergonier *et al.*, 2003; Contreras *et al.*, 2003). However, the pathogenicity of CNS, either as a group or as individual species, is not well described. Most studies have found no significant association between CNS IMI and milk yield (Moroni *et al.*, 2005*a*; Koop *et al.*, 2010), although Leitner *et al.* (2004*a*) reported a lower milk yield in infected halves than in contralateral uninfected halves. Studies on the association between udder-half-level species-specific CNS IMI and milk somatic cell count (SCC) have also yielded conflicting results (Deinhofer and Pernthaner, 1995; Moroni *et al.*, 2005*a*; Koop *et al.*, 2012). Conflicting data on the association of CNS IMI with SCC may, in part, be explained by the fact that goats' milk SCC can be affected by factors other than bacterial IMI such as parity, days in milk (DIM), and infection with small ruminant lentivirus (SRLV) (Sanchez *et al.*, 2001; Haenlein, 2002). Moreover, age, DIM and, possibly, SRLV infection can potentially affect acquisition of a new IMI in dairy goats (Contreras *et al.*, 1999; Moroni *et al.*, 2005*b*; McDougall *et al.*, 2014), and should be considered confounding factors (Rothman *et al.*, 2008) when evaluating relationships between IMI and SCC in goats.

The persistence of an IMI may indicate host-adaptation. In the few studies describing the persistence of CNS IMI in goats, *Staphylococcus chromogenes*, *Staphylococcus xylosus*, *Staphylococcus epidermidis* and *Staphylococcus caprae* were the most common species causing persistent IMI (Contreras *et al.*, 1997; Moroni *et al.*, 2005*a*; Koop *et al.*, 2012). In these studies, the diagnosis of a persistent IMI was based on detection of the same CNS species on more than one consecutive sampling. However, because these CNS species are also those with the highest relative prevalence, a new infection by a different strain of the same species following cure is also possible, leading to a false conclusion about the persistence of the IMI, as has been reported in cows (Fry *et al.*, 2014). The latter occurrence can only be ruled out by identifying bacterial isolates at the sub-species level using, for instance, strain-typing to demonstrate that the same species and strain-type are present in consecutive milk samples over time.

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In a previous, single point-in-time, observational, cross-sectional study by our research group, we reported that goats with *S. chromogenes* isolated from a single composite milk sample had a higher goat-level somatic cell score (SCS) than goats with no growth from a single composite milk sample (Bernier Gosselin *et al.*, 2018). However, goat-level SCS is likely to be an imprecise predictor of the inflammation associated with different staphylococcal species because of the potential dilutional effect of an uninfected udder-half on the goat-level SCS. Therefore, the objectives of the present study were to build on our previous study by conducting a second study in the same herd to (1) describe, using strain-typing data, the persistence of species-specific CNS IMI at the udder-half level over a 2-month period in goats at various stages of lactation, and (2) evaluate the relationship among species-specific CNS IMI, no growth and SCS at the udder-half level.

Materials and methods

Herd

The herd studied had 909 lactating goats in year-round production. Goats were of mixed genetic lineages including cross-bred Saanen, Alpine, Toggenburg, and La Mancha animals. The goats were housed in five separate pens containing 83 to 229 goats per pen. Average annual milk production approximated 900 kg per animal. The goats were machine-milked twice daily. The milking time hygiene protocol included pre-milking spray disinfection using an iodine-based product, drying of the teats with a clean single-use cloth, post-milking teat dipping and wearing of gloves by the milking personnel.

Sampling and cell counting

The study protocol was approved by the University of Missouri Animal Care and Use Committee. The study design was an observational longitudinal study. All 909 goats had udder-half foremilk samples ($n = 1817$ halves; one goat had a non-functional udder-half) collected aseptically for culture and SCC at the beginning of the study (Day 0) as described by Bernier Gosselin *et al.* (2018), with the exception that milk was collected from each udder half into separate, sterile plastic tubes. Each goat's identification number and tube number were recorded. Milk samples for culture were frozen at −20 °C until analysed. Udder-half milk samples for cell counting were collected into separate vials containing a 2-bromo-2-nitropropane-1,3-diol preservative tablet (Broad Spectrum Microtabs II, D & F Control Systems, Inc., Dublin, CA, USA), refrigerated and shipped overnight to a commercial laboratory (Mid-South Dairy Records, Springfield, MO, USA) within 2 d of sample collection for cell counting using an automated counter (Bentley Somacount FCM, Bentley Instruments, Chaska, MN, USA). Results were reported to the investigators electronically.

Follow-up udder-half milk samples for culture and SCC were collected likewise at approximately monthly intervals (Day 37 and Day 61) from goats diagnosed with a CNS IMI (see below) in one or both halves on Day 0.

Bacterial culture

Bacterial culture of milk samples was performed on Columbia blood agar (CBA) as described in Bernier Gosselin *et al.* (2018). Colonies were identified based on morphology, catalase test and coagulase test according to National Mastitis Council guidelines (Middleton *et al.*, 2017). Udder-halves whose milk sample yielded ≥1 colony (~100 cfu/ml) of a single, catalase positive, coagulase negative colony on CBA at 48 h were presumptively identified as a CNS IMI. Udder-halves with milk samples that yielded two different colony types were defined as mixed IMI. Udder-halves with milk samples that yielded ≥3 colony types were defined as contaminated. Presumptively identified CNS isolates were subcultured on CBA and stored in phosphate-buffered glycerol at −80 °C for further identification. Isolates presumptively identified as coagulase-positive *Staphylococcus* spp., or another genera, were not stored or further characterised.

Species identification

Presumptive CNS isolates were grown on CBA and identified to the species-level using matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry as described by Bernier Gosselin *et al.* (2018). Briefly, identification was performed in two steps, using the direct transfer method in duplicate and complemented by the ethanol and formic acid extraction method when identification by direct transfer was unsuccessful. An isolate was considered successfully identified when a score of ≥2.0 was assigned to a given species (Tomazi *et al.* 2014) by the proprietary software (Bruker Daltonics, Billerica, MA, USA) using the manufacturer's isolate identification database.

When identification with MALDI-TOF was unsuccessful, isolates were prepared for PCR amplification and partial gene sequence analysis of either *tuf* or *rpoB*. Lysate preparation for PCR, purification of PCR products, DNA sequencing and species identification assignment were conducted as described by Bernier Gosselin *et al.* (2018). Polymerase chain reaction amplification and sequence analysis of *tuf* were performed first (Hwang *et al.* 2011). When amplification or sequencing with *tuf* was unsuccessful or did not meet identification criteria (i.e. ≥98% sequence similarity and >0.8% separation from the next species), amplification and sequence analysis of *rpoB* was performed (Drancourt and Raoult, 2002). If amplification or sequence analysis was unsuccessful (i.e. ≥97% sequence similarity), DNA extraction was performed using a DNeasy kit (Qiagen, Valencia, CA, USA), then amplification and sequence analysis of *tuf* or *rpoB* was repeated, as applicable. Isolates that were not successfully identified after DNA extraction and PCR amplification and sequence analysis were deemed unidentified.

Strain-level identification

When isolates of the same species were recovered from the same udder-half on more than one occasion, isolates from the first and last sample were subjected to strain-level identification by pulsed-field gel electrophoresis (PFGE) as described previously (Middleton *et al.*, 2002). Pairs of isolates of the same species from the same udder-half were placed side by side in the gel and their PFGE banding patterns compared after staining the electrophoretogram with ethidium bromide and visualising the results with ultraviolet transillumination. Isolates with the same number of bands of the same molecular weights based on visual assessment were considered the same strain (i.e. 100% similarity). If an udder-half had the same strain isolated on Days 0 and 61, and was positive for the same species on Day 37, it was assumed that all isolates were the same strain. Additionally, when a mixed

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IMI was diagnosed on Day 61, isolates of the same species as Day 0 were strain-typed. When the isolates from the same udder-half yielded different strains on Days 0 and 61, isolate(s) from Day 37 were strain-typed and compared with the isolate from Day 0.

Statistical analyses

Somatic cell score was computed using the equation: SCS = ln (SCC/100)/0.6931 + 3 (Hogan *et al.*, 1999). Further, parity data were dichotomised as 1 or >1.

Relationship between CNS species and odds of IMI persistence

Only udder-halves that had a CNS IMI on Day 0 and culture results on Days 37 and 61, with species identification and strain type when applicable, were included in the analysis. Based on culture of the three samples and PFGE results, an IMI was defined as persistent if the same strain of the same species isolated on Day 0 was isolated on ≥1 follow-up sample, i.e. on Days 37 and/or 61 (categories B, C and D in Table 1). When an IMI was only detected at the first sampling and followed by two samples negative for the same species and strain, the IMI was considered non-persistent (category A in Table 1). When a mixed IMI was identified on Days 37 or 61, the original CNS IMI was considered persistent if one of the isolates from the mixed IMI was of the same species and strain as the isolate from Day 0. Among all halves with a CNS IMI on Day 0, the proportion of persistent IMI was compared among CNS species. To improve model convergence, all species with ≤10 IMI were grouped together into a single category defined as 'other CNS'. Hence, species were categorised as *S. caprae*, *S. epidermidis*, *S. xylosus*, *Staphylococcus arlettae*, *Staphylococcus simulans* and other CNS (i.e. *S. chromogenes*, *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lentus* and *Staphylococcus warneri*).

Bivariate analyses were performed to evaluate the odds of persistence as a function of CNS species IMI. A generalised mixed linear model was then used to evaluate the odds of persistence as a function of CNS species IMI after adjusting for DIM and parity, and with a random effect at the goat level. Tukey adjustment was used when comparing least square means (LSM) of different CNS species.

Relationship between IMI status and SCS

Data included in the analyses of the relationship between IMI status and SCS are shown in online Supplementary Fig. S1. Culture results and SCS data were considered at the level of observations recorded on a given day, regardless of persistence status of the half. For example, a *S. chromogenes* IMI persisting over 61 d contributed three *S. chromogenes* observations, whereas a non-persistent *S. equorum* IMI contributed one *S. equorum* observation and two no-growth observations. However, when an udder-half had a same strain recovered from Days 0 and 61 but not from Day 37, the culture result on Day 37 was considered a false-negative and the half was considered infected with that CNS species on Day 37. Mixed IMI (Days 37 or 61) caused by different species were excluded from the analysis. To improve model convergence, all species with ≤10 observations were grouped together into a single category defined as 'other CNS'. Species were categorised as *S. caprae*, *S. chromogenes*, *S. epidermidis*, *S. xylosus*, *S. arlettae*, *S. simulans* and other CNS (i.e. *S. capitis*, *S. cohnii*, *S. equorum*, *S. haemolyticus*, *S. hominis*, *S. lentus* and *S. warneri*). The other category in this context differs from the previous analysis due to the number of observations differing from the number of defined IMI, e.g. *S. chromogenes* accounted for <10 IMI, but because all IMI persisted, >10 discrete observations were recorded for *S. chromogenes* IMI in the data set.

A mixed linear model was built to evaluate the effect of IMI status (CNS category or no growth) on SCS, after adjusting for confounding by DIM and parity, with random effects at the half and goat levels. Unconditional relationships between the independent variables and the outcome were first evaluated, and linearity of the relationship between DIM and SCS was assessed using polynomial square and cubic terms. Polynomial terms that were statistically significant were kept for inclusion in the model. Interactions between IMI status and DIM or parity were assessed. Significance was set at $P < 0.05$. Normality and homoscedasticity of residuals were visually assessed. Tukey adjustment was used for comparing SCS of halves with different IMI status. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

Of the 909 goats (1817 halves; 1 goat had a non-functional udder-half) available at the initial sampling (Day 0; online Supplementary Fig. S2), six goats had a single web teat and one goat had an injured teat. These udder-halves ($n = 7$ halves) were excluded. A further 27 goats (54 halves) with missing or duplicate identification numbers were also excluded. From the remaining 882 goats (1756 halves), 926 udder halves yielded no growth, 252 halves had a single CNS IMI, 158 halves had a mixed IMI, 168 halves had a non-staphylococcal IMI, 11 halves had a coagulase positive staphylococcal IMI and 241 halves yielded contaminated samples. Consequently, 252 halves from 220 goats with a CNS IMI were selected to be sampled again 37 and 61 d after the initial sampling.

Overall, a total of 690 CNS isolates were characterised, of which 90.9% (627 isolates) were assigned a species identification with MALDI-TOF using a score of ≥2.0. For the remaining isolates, 88.9% (56/63) were identified by partial gene sequencing. Seven isolates could not be speciated using MALDI-TOF or housekeeping gene sequence.

Among the CNS IMI that persisted based on species-level identification, 6.9% (10/144) had different strains on Days 37 and 61 and were thus defined as non-persistent. Applying the results of PFGE, 9.4% (6/64) of *S. simulans*, 3.1% (1/32) of *S. xylosus* IMI, 5% (1/20) of *S. caprae* IMI, 9.1% (1/11) of *S. epidermidis* IMI and 50% (1/2) of *S. cohnii* IMI that were suspected to be persistent based on species identification alone, were defined as non-persistent based on PFGE. Conversely, 100% of IMI caused by *S. chromogenes* ($n = 10$), *S. warneri* ($n = 3$) and *S. arlettae* ($n = 1$) that were considered persistent based on species identification were confirmed to have the same strain-type and thus defined as truly persistent. Overall, 69.8% (134/192) CNS IMI persisted, of which 130 were confirmed to persist over the 61 d study period (categories C and D in Table 1). The numbers of IMI classified based on culture results of each sample and PFGE are presented in Table 1, and the numbers and proportions of persistent IMI, by species, are presented in Table 2.

Relationship between CNS species and odds of IMI persistence.

When appraising relationships between CNS species and odds of an IMI persisting, 192 halves (from 170 goats) with CNS IMI on

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Table 1. Distribution of the four intramammary infection (IMI) categories based on culture results of three consecutive udder half-level milk samples at approximately 1-month intervals

Category	Day 0	Day 37	Day 61	Number of IMI
A	Positive	Negative[a]	Negative[a]	58
B	Positive	Positive	Negative[a]	4
C	Positive	Negative[a]	Positive	6
D	Positive	Positive	Positive	124

[a]Includes no growth, different species and different strains than on Day 0.
Note: Category A was defined as non-persistent and categories B, C and D were defined as persistent.

Day 0 (134 persistent and 58 non-persistent) were included in the model. Goats' median (range) parity was 2 (1 to 5), with 44.1% goats of first parity. Median (range) DIM on the initial sample was 130 (13 to 912) d. In bivariate analyses, DIM ($P = 0.86$) and parity ($P = 0.62$) were not significantly associated with the odds of persistence, but were still included in the final model as confounders of the relationship between CNS species and odds of IMI persistence. The reference categories used in the model were parity 1 and *S. simulans* for the CNS species variable, due to its high prevalence and high proportion of persistent IMI. The final model showed that CNS species was associated with the odds of IMI persistence ($P = 0.03$; online Supplementary File – Table S1). After adjustment for multiple comparisons, *S. simulans* IMI had higher odds of persistence (OR 41.7, $P = 0.04$) compared with *S. arlettae* IMI, and *S. caprae* IMI tended (OR 50, $P = 0.05$) towards a higher odds of persistence compared with *S. arlettae* IMI.

Relationship between IMI status and SCS.

After exclusion of five mixed species IMI, and one non-staphylococcal IMI, 1496 observations (from 725 goats) were eligible for inclusion in the model (online Supplementary Fig. S1), with 1118 halves from Day 0, 187 halves from Day 37 and 191 halves from Day 61. Due to missing data for SCC ($n = 11$) or parity and DIM ($n = 26$), 1469 observations were included in the final model. The median (range) parity was 2 (1 to 6), with 41.7% goats being first parity. Median (range) DIM on Day 0 was 124 (3 to 912) d. Overall, a mean SCS of 6.2 was observed with a range of 0.06 to 9.6, corresponding to a geometric mean SCC of 919 000 cells/ml and range of 13 000 to 9 695 000 cells/ml. The final model showed that SCS was associated with IMI status ($P < 0.01$) after adjusting for confounding by parity and DIM (online Supplementary File – Table S2). Moreover, the effect of IMI status on SCS was shown to vary as a function of parity (IMI status × parity; $P < 0.01$). Variation of SCS by IMI status and parity is shown in Fig. 1. After adjustment for multiple comparisons, for halves from goats of parity 1, SCS of *S. caprae*, *S. epidermidis* and *S. simulans* IMI were significantly higher than for halves with no growth, whereas for parity >1 only the SCS of *S. simulans* IMI was significantly higher than no growth. Additionally, for parity 1, the SCS of *S. epidermidis* IMI was significantly higher than *S. arlettae*, *S. xylosus* and other CNS IMI (Table 3).

Discussion

With the exception of *S. arlettae* and *S. cohnii*, most of the species causing persistent IMI in the present study have been reported previously to cause persistent IMI, although that persistence has been based on species-level identification only without confirmation by strain-typing (Poutrel, 1984; Contreras *et al.*, 1997). Others have reported that *S. caprae*, *S. chromogenes*, *S. xylosus*, *S. simulans* and *S. epidermidis* are the most common CNS species to cause IMI in goats and also the most common species to cause persistent IMI (Contreras *et al.*, 1997; Leitner *et al.*, 2007; Koop *et al.*, 2012). In the present study, *S. simulans* was the most prevalent species and frequently caused persistent IMI. Because the relative prevalence of CNS species varies between herds (Contreras *et al.*, 1995; Koop *et al.*, 2012), a measure of association may be more useful than a measure of frequency. In the present study, the model evaluating the odds of persistence of IMI identified CNS species as a significant factor. More specifically, *S. simulans* were significantly associated with higher odds of persistence than *S. arlettae*. The inclusion of only one herd was a limitation of this study, since different species distributions in other herds could have resulted in different CNS species groups. While the results were from a single herd and it is unknown whether they would be replicated in other herds, these data are corroborated by other studies. Further, the addition of strain-typing to characterise IMI in this study added credence to previous reports that only used species-level identification to define IMI persistence as the current study suggested that only a minority of IMI would be misclassified as persistent by using species identity alone.

The mixed linear model showed a significantly higher SCS in halves with *S. caprae*, *S. epidermidis* or *S. simulans* IMI than uninfected halves, which is in agreement with studies by others

Table 2. Number and proportion of intramammary infections (IMI) that were persistent or not based on strain-level identification of isolates listed according to staphylococcal species

Species	Number (%)[a] of same-strain IMI		Total
	Not persistent[b]	Persistent[b]	
Staphylococcus simulans	14 (19.4)	58 (80.6)	72
Staphylococcus xylosus	13 (29.5)	31 (70.5)	44
Staphylococcus caprae	4 (17.4)	19 (82.6)	23
Staphylococcus epidermidis	4 (28.6)	10 (71.4)	14
Staphylococcus arlettae	10 (90.9)	1 (9.1)	11
Staphylococcus chromogenes	0	10 (100)	10
Staphylococcus lentus	7 (87.5)	1 (12.5)	8
Staphylococcus equorum	2 (100)	0	2
Staphylococcus warneri	0	3 (100)	3
Staphylococcus cohnii	1 (50)	1 (50)	2
Staphylococcus capitis	1 (100)	0	1
Staphylococcus haemolyticus	1 (100)	0	1
Staphylococcus hominis	1 (100)	0	1
Total	58 (30.2)	134 (69.8)	192

[a]Percentage of persistent or non-persistent IMI within row.
[b]Persistence was defined as IMI yielding same species and strain (based on PFGE) isolates from Day 0 and from Days 37 or 61.

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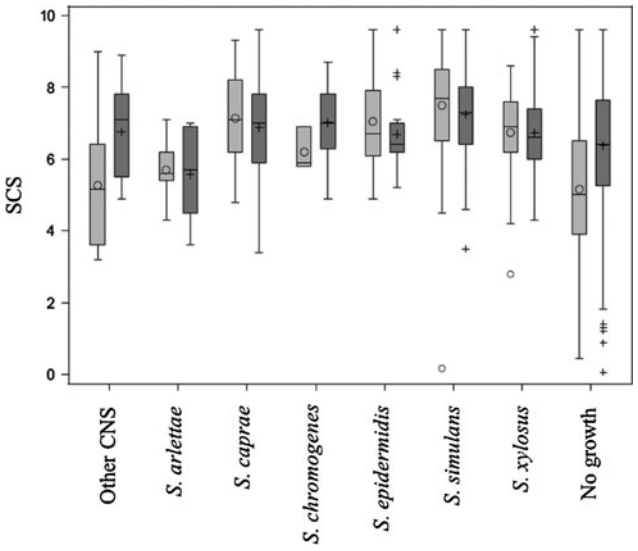

Fig. 1. Box plot of half-level somatic cell score (SCS) by intramammary infection (IMI) status and parity. For each IMI status, the pale grey (left) box represents primiparous goats and dark grey (right) represents multiparous goats. Other coagulase negative staphylococci (CNS) included *S. capitis* ($n = 1$), *S. cohnii* ($n = 7$), *S. equorum* ($n = 2$), *S. haemolyticus* ($n = 1$), *S. hominis* ($n = 1$), *S. lentus* ($n = 9$) and *S. warneri* ($n = 9$).

Table 3. Least squares mean and 95% confidence intervals (CI) for somatic cell score (SCS) of halves with a given intramammary infection status and parity computed using results from the mixed model

	Parity 1		Parity >1	
Effect	Estimate[a]	95% CI[a]	Estimate[a]	95% CI[a]
S. epidermidis	7.9[b]	7.1–8.8	7.8[d, e]	6.8–8.7
S. chromogenes	7.7[a, b,c]	5.7–9.7	6.9[d, e]	6.1–7.6
S. simulans	7.0[b, c]	6.6–7.4	7.2[e]	6.8–7.6
S. caprae	6.3[b, c]	5.7–7.0	7.0[d, e]	6.4–7.7
S. xylosus	6.0[a, c]	5.5–6.5	6.8[d, e]	6.4–7.3
Other CNS [b]	5.7[a, c]	5.0–6.4	6.6[d, e]	5.8–7.4
S. arlettae	5.3[a, c]	4.3–6.4	6.4[d, e]	5.5–7.4
No growth	5.2[a]	5.0–5.4	6.4[d]	6.3–6.6

[a]The estimates represent SCS for a goat at 150 d in milk (DIM), which was the reference category in the model. For example, for a goat at 250 DIM (100 more DIM than the reference), based on the estimates in Supplementary Table S1 for DIM (0.005) and DIM squared (-7.45×10^{-6}), $100 \times 0.005 + 100^2 \times -7.45 \times 10^{-6} = 0.4255$ would be added to the estimate. Conversely, for a goat at 50 DIM, 0.4255 can be subtracted from the estimate.
[b]Other coagulase negative staphylococci (CNS) included *S. capitis* ($n = 1$), *S. cohnii* ($n = 7$), *S. equorum* ($n = 2$), *S. haemolyticus* ($n = 1$), *S. hominis* ($n = 1$), *S. lentus* ($n = 9$) and *S. warneri* ($n = 9$).
Note: In the same parity group (within column), estimates with different superscripts are significantly different, after Tukey adjustment for multiple comparisons.

(Deinhofer and Pernthaner, 1995; Sanchez *et al.*, 1999; Moroni *et al.*, 2005*a*); however, the difference in SCS was only observed in primiparous goats in the present study. Conversely, in multiparous goats, only SCS associated with *S. simulans* IMI was significantly different from uninfected halves. When comparing CNS species, some authors report little or no difference in SCS (Contreras *et al.*, 1999; Leitner *et al.*, 2004*b*; Koop *et al.*, 2012), whereas Moroni *et al.* (2005*a*, 2005*b*) reported *S. caprae* were associated with a higher SCS than other CNS, except *S. epidermidis*. The model presented here showed *S. epidermidis* to be associated with a significantly higher SCS than *S. arlettae*, *S. xylosus* and other CNS in primiparous goats, whereas no differences were detected in multiparous goats. To the authors' knowledge, an effect of the interaction of IMI status by parity on SCS has not been previously reported. The interaction of IMI status and parity may be due to differences in the immunity of the mammary gland between primiparous and multiparous goats, which is further supported by the well-described relationship between goat-level milk SCC and increasing parity (Wilson *et al.*, 1995; Paape *et al.*, 2007). Moroni *et al.* (2005*a*) reported that the early-to-mid stages of IMI are associated with the largest increase in SCS. Different degrees of chronicity of IMI between primiparous and multiparous goats may have affected SCS differently in the present study. For example, some IMI in multiparous goats may have been carried over more than one lactation. Additionally, SRLV infection may affect SCS (Ryan *et al.* 1993; Sanchez *et al.* 2001) and this effect may vary with age. For the goats in the present study, SRLV serology status and history of IMI or SCC were unknown and hence associations of SRLV with SCS were not evaluated. True duration of IMI could not be included in the persistence model because the time when IMI was acquired was unknown. Similarly, most IMI persisted until the end of the study, precluding estimation of the time to elimination.

Strain-typing of isolates using PFGE identified 10 same-species IMI that would have been classified as persistent based on species identification alone, but were revealed to be an IMI with a different strain of the same species in the third sample. To the authors' knowledge, strain typing has not been previously used to determine the persistence of CNS IMI in goats. Given the high prevalence of CNS IMI in goats, mammary glands with an IMI caused by a given species may eliminate the IMI and become infected with a new strain of the same species at a subsequent sampling. While not common, the present study documented that this occurs, and the occurrence rate was similar to that reported for CNS IMI in cows (Fry *et al.*, 2014). Although persistence was ruled out in 10 IMI based on PFGE, one limitation of this study was that strain characterisation was based on only one colony isolated from the initial milk culture (Muellner *et al.*, 2016), which may have affected the diagnostic sensitivity for persistent IMI if a mixed microbial population of strains happened to be present in the sample.

In conclusion, the most prevalent species isolated from udder-half milk samples were *S. simulans*, *S. xylosus*, *S. caprae* and *S. epidermidis* and these species tended to cause persistent IMI. *Staphylococcus arlettae* was associated with lower odds of persistence than *S. simulans*, and had a lower SCS than *S. epidermidis*, which suggests it may be less pathogenic than some of the other CNS species. These data provide evidence of potential differences in pathogenicity between CNS species isolated from goat milk, and further investigation of more herds over a longer period of time will help further define the differential pathogenicity (SCS, persistence and reservoirs) of CNS to help identify which CNS species should be targeted in udder health programmes. The study of persistence of CNS IMI in goats should include the use of strain-typing, as same-species IMI caused by different strains over time may occur; however, in routine herd management, strain-typing may not be necessary.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000311.

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