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Somatic cell count in dairy goats II: udder health monitoring at goat and herd level

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Abstract

Background Mastitis is a costly disease affecting dairy ruminants worldwide. Somatic cell count is the most widely used tool for udder health monitoring but is highly influenced by non-infectious factors in goats. The aim of this paper was to define adjusted action thresholds of somatic cell count for the detection of goats with intramammary infections. A secondary aim was to investigate the potential of bulk milk analyses in identifying herds with udder health issues. Nine Norwegian dairy goat herds were visited five times during one lactation. Somatic cell count measurements combined with bacteriological testing were performed both at goat and herd level.

Results *Staphylococcus (S.) aureus* was the udder pathogen with the strongest influence on the somatic cell count and was therefore selected as the focus of udder health control. The period prevalence of *S. aureus* in the included herds varied from 1 to 40%. The thresholds for identifying goats with *S. aureus* intramammary infection varied from 500,000 cells/mL in first parity goats in early lactation to 3 million cells/mL in goats of higher parities on pasture. A herd prevalence of *S. aureus* of more than approximately 10% was associated with significant bulk milk quality challenges.

Conclusions The study defined adjusted action thresholds of somatic cell count for the detection of intramammary infections in dairy goats. The somatic cell count levels in goats with *S. aureus* were in most cases well distinguished from those with no or minor pathogen findings when adjusting according to parity, pasture-status, and lactation stage. The patterns of routinely measured bulk milk parameters, along with analyses of bacteriological composition, provided a rough indication of the herd's udder health status.

Keywords Mastitis, Milk quality, Parity, *S. aureus*, *Spa* type, Total bacterial count

Background

Intramammary infections (IMI) affect milk quality and animal welfare and are a leading cause of economic losses in the dairy industry [1]. Early detection of udder health problems is essential to limit these consequences. Somatic cell count (SCC) is an important udder health indicator, providing a valuable tool for recognition, surveillance, and prevention of IMI [2, 3]. The SCC action thresholds are well-defined in dairy cows both on individual and herd level [4, 5]. Goat milk generally has higher SCC than cows' milk, and the reasons for this are not completely understood. Goat milk has a higher number of neutrophils [6–8], and some studies found that epithelial cells can make up to 50–70% of the cell population [7, 9, 10]. Additionally, SCC in goat milk is significantly influenced by non-infectious factors including stage of lactation, breed, parity, and management [11–14]. For

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these reasons, the SCC thresholds applied in cows cannot be used in goats, and thresholds differentiating infected udders from uninfected are poorly defined for goat milk.

Somatic cell count is measured in individual goat milk in herds participating in dairy herd improvement (DHI) programs. The SCC is used as a screening tool, where goats suspicious of having an IMI are further sampled to confirm or rule out IMI [2, 15]. Preferably, the pattern from several SCC measurements combined with bacterial diagnostics provide the basis for udder health decisions for that animal, including segregation, sampling, treatment, or culling. Bacterial culture or quantitative polymerase chain reaction (qPCR) are the most widely used tests to confirm IMI [15, 16].

Measurement of SCC in bulk milk is performed routinely by most dairies, and payment based on SCC is used to stimulate a hygienic production of milk from healthy animals. Another measure of the hygienic quality of milk is the total bacterial count (TBC). The bulk milk SCC and TBC may also have potential as indicators of the herd udder health [4, 16, 17]. However, high TBC may also originate from sources other than IMI, e.g. general hygiene, or issues with the cleaning of the milking machine or milk tank. Additional analyses of the bacterial composition of bulk milk may supplement the routinely measured parameters to identify possible sources of high TBC. Analyses of bulk milk samples have also been found useful in detecting udder pathogens that have a significant presence in a herd [18, 19]. Goat milk production involves a high number of individuals producing a relatively low amount of milk, which makes milk recordings relatively more laborious than in cows. Although bulk milk analyses may be imprecise, they are more easily available than individual goat samples, which makes it important to elucidate the potential of bulk milk diagnostics in goat milk production.

Norway has approximately 240 goat milk farms producing a total volume of approximately 18 million liters of goat milk [20]. The main breed is the Norwegian dairy goat, and the average herd size is 138 lactating goats. The goat milk production is seasonal, with most herds having concentrated kidding in the period between January to April. The goats are housed in the winter and grazed, usually on mountain pastures, during the summer. All dairy goat herds in Norway participated in the sanitation program "Healthier goats" in the period 2001 to 2014 where caprine arthritis encephalitis (CAE), caseous lymphadenitis (CLA) and paratuberculosis were eradicated [21]. Mycoplasma mastitis has never been detected in Norwegian dairy goats. After eradicating important chronic diseases from the dairy goat population, mastitis is probably the costliest infectious disease of dairy goats in Norway, although the exact prevalence of mastitis is unknown due to incomplete recordings regarding treatments and culling related to mastitis.

Staphylococcus (S.) aureus is the most important udder pathogen influencing goat milk quality in Norway and the primary focus of udder health control programs [22]. *S. aureus* is generally considered a contagious udder pathogen, and subclinically infected goats are important sources of *S. aureus* causing clinical mastitis [23]. The ability of *S. aureus* to spread within a herd varies, possibly due to differences in management factors or bacterial strains [24, 25].

Healthy udders lead to more efficient production as well as better milk quality and animal welfare. To address udder health problems at an early stage, the farmers and their advisory teams need proper tools to recognize them. Defining thresholds for unhealthy udders or herds based on SCC in dairy goats is challenging because of the large influence of non-infectious factors like parity, lactation stage and season, as shown in the first part of this study [26], as well as in several other studies (e.g. [12, 27, 28]). This can be solved by adjusting the thresholds for known influential physiological factors [29]. The primary objective of the present paper was to explore adjusted thresholds for SCC in udder health management and surveillance in dairy goat herds, with a special focus on *S. aureus* control. A secondary objective was to assess the potential of bulk milk testing as a tool for monitoring udder health in dairy goat herds.

Results

Study population and period

Nine Norwegian dairy goat farms (farm A-I) were visited in five sampling events during one lactation between March and November 2022. Table 1 provides an overview of the samples and analyses leading to the results presented in this section and how they relate to the objectives of the study.

The farms were located in the east (farm A-E), west (farm F and G) and northern (farm H and I) parts of Norway, and the median herd size was 98 (range 65–192) lactating goats. All herds were of the Norwegian milk goat breed and certified free from CAE, CLA and paratuberculosis.

Goat level findings

The dataset included 4403 observations from milk recordings and 4197 observations with bacteriology results. Of these, 555 observations were incomplete due to missing or incorrect numbering of samples from individual goats in either milk recordings or bacteriology sampling. In total 3848 observations were successfully merged to complete observations with results from milk recording and bacteriology. None of the goats had

Table 1 Overview of samples and analyses in the study and relation to the study objectives

	Sampling level	Collection	Analyses	Objectives
Milk recording samples	Goat	Sampling 1–5, all lactating goats	Somatic cell count	Define action thresholds for identification of goats with intramammary infection
Aseptically obtained milk samples	Udder half	Sampling 1–5, all lactating goats	Bacterial culture *Spa* typing of selected *Staphylococcus aureus*	*Staphylococcus aureus* control: Bacterial identification and subtyping
Bulk milk samples (routine)	Bulk milk	Routinely at milk collection every third day	Somatic cell count Total bacterial count	Investigate the potential of bulk milk analyses as a method for identifying herds with udder health issues
Bulk milk samples for bacteriological composition	Bulk milk	Sampling 1–5	Bacterial culture qPCR	

clinical mastitis at the time of sampling. The mean daily milk yield was 2.62 (SD 0.98) liters. Seventeen observations with contamination on bacteriology were excluded, resulting in 3831 goat-level samples (7662 udder half samples for bacteriology) from 767 goats in the descriptive statistics.

At the udder half level, 5981/7662 (78%) of samples were culture negative. Among culture positive samples, one pathogen only was detected in all samples except for 26/7662 (0,3%) samples reported as contaminated due to growth of more than two colony types. The most frequent findings were different non-aureus staphylococci and mammaliicocci (NASM); *S. epidermidis*, *S. caprae*, and *S. warneri*, identified in 461/7662 (6%), 430/7662 (6%), and 313/7662 (4%) of the samples, respectively. *S. aureus* was identified in 231/7662 (3%) of udder halves. Among these 231 *S. aureus*-detections, 47 (20%) showed sparse growth (100–500 colony forming units [cfu]/mL). In seven udder halves (from six goats) the *S. aureus* was anhaemolytic, all of which were confirmed as *S. aureus* with MALDI-ToF. Other bacteria identified (less than 1% of udder halves) were *S. simulans* ($n=41$), *Pseudomonas* sp ($n=28$), *S. chromogenes* ($n=12$), NASM other than the abovementioned ($n=19$), *Corynebacterium bovis* ($n=12$), and *Corynebacterium* sp ($n=10$). Important udder pathogens like *Escherichia coli* were detected at goat level in five samples, *Streptococcus (Str.) dysgalactiae* in two samples and *Str. uberis* in one sample. The prevalence of the most common bacteriology findings at each farm is presented in Additional file 1 (Figure A1). The SCC and period prevalence for the most frequent goat-level bacteriology findings are presented in Table 2. The ln-transformed SCC (lnSCC) was significantly higher in observations with bacterial status *S. aureus* than other bacterial findings (Fig. 1).

For further analysis, the observations were classified as *S. aureus*-positive or *S. aureus*-negative ("*S. aureus* status"). Two hundred and ten observations were *S. aureus*-positive, of which 135/210 (64%) had *S. aureus* in one udder half and a culture negative contralateral udder half, 18/210 (9%) had *S. aureus* in both udder halves, and 47/210 (22%) had *S. aureus* in one udder half and NASM (in pure culture, ≥ 500 cfu) in the contralateral udder half. The mean SCC and standard deviation (SD) in the three groups were 3210 (SD = 2844), 3566 (SD = 3163), and 2038 (SD = 1857) × 1000 cells/mL, respectively. The SCC according to *S. aureus* status varied greatly with respect to parity and sampling period (Table 3). Of the *S. aureus* positive observations, 54 of 210 (26%) had SCC lower than 1 million cells/mL, the current recommended reaction threshold for *S. aureus* detection of goats in Norway. These 54 observations included mainly observations from first ($n=16$, 30%) or second ($n=20$, 37%) parity goats, and 54% of the observations were in early lactation (indoor). A high proportion (26/54, 48%) of these samples had sparse growth of *S. aureus,* and in 28 of 54 (52%) samples, *S. aureus* was not detected at the next sampling event and was therefore considered transient findings. Of the transient findings, 22/28 (79%) had sparse growth of *S. aureus.* When excluding the observations with sparse growth of *S. aureus*, most goats (> 75%) had SCC above one million cells/mL (Additional file 1, Table 1).

The prevalence of *S. aureus* at each sampling varied from 0–33% between farms and samplings (Table 4). Altogether 108 of the 767 goats had *S. aureus* detected at least once, giving an overall period prevalence of 14%.

A total of 44 *S. aureus* isolates were *spa*-typed. Altogether 12 *spa* types were detected, among which one was not reported previously. Between one and three *spa* types were identified per farm. *Spa* type t2678 was the most common finding, identified in four herds from two different geographical regions (Table 4).

Herd level findings

Routine bulk milk analyses

Results from the routine analyses of bulk milk SCC and TBC for the eight farms delivering milk to the

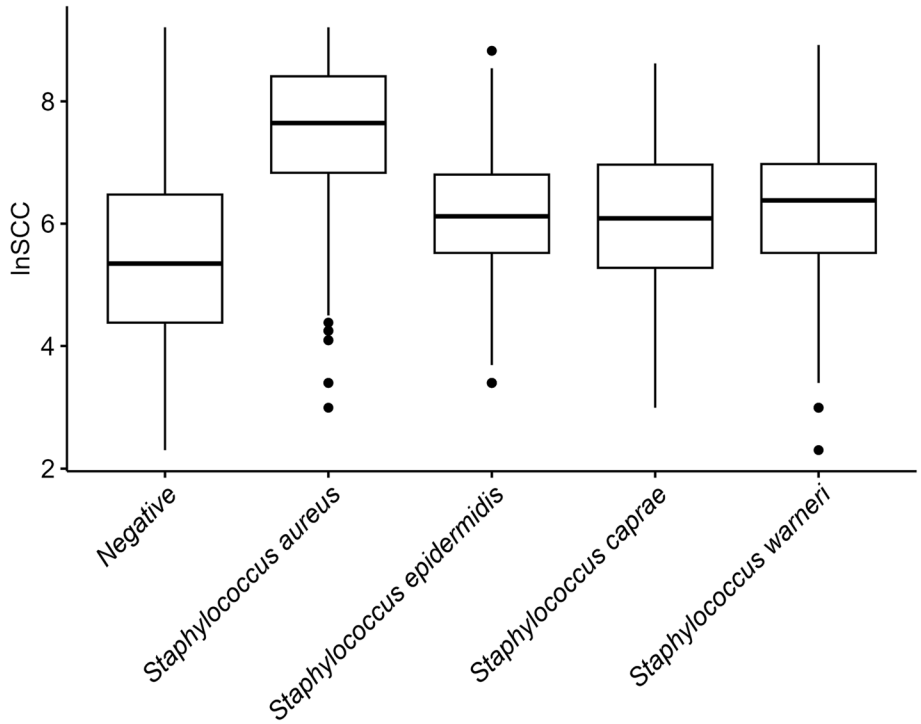

Fig. 1 Distribution of lnSCC according to goat-level intramammary infection status. The data is based on 3848 observations of 767 goats from nine Norwegian dairy goat herds

Table 2 Somatic cell count (×1000 cells/mL) and period prevalence according to goat level bacteriological status in 767 goats from nine Norwegian dairy goat herds sampled up to five times during one lactation

	Somatic cell count				
Bacterial status	**Median**	**Interquartile range**	**n observations**	**n goats**	**Prevalence (%)[a]**
Staphylococcus aureus	2090	928—4488	210	108	14
Staphylococcus caprae	440	196—1059	285	132	17
Staphylococcus epidermidis	455	250—900	324	128	17
Staphylococcus warneri	590	250—1070	185	111	14
Negative	210	80—649	2504	641	84

[a] Period prevalence, calculated as the proportion of goats with the bacterial status in at least one sampling event during the study period

Norwegian dairy company, TINE, are plotted in Fig. 2 and described in Table 5. The marked increase in bulk milk SCC seen in all farms except D and F (Fig. 2) was associated with the herd being released to pasture.

The herds with more than 10% of the goats with *S. aureus* IMI (farms B, G, and early lactation I) were characterized by a bulk milk SCC higher than 800,000 cells/mL in early lactation (Table 5) and several peaks of higher bulk milk TBC (Fig. 2).

Farm F had a remarkably low bulk milk SCC and elevated TBC during the study period (Fig. 2/Table 5). The udder health in this herd was considered good, with a very low prevalence of *S. aureus*, and a prevalence of NASM comparable with the other herds (Additional file 1, Figure A1).

Investigation of bacteriological composition of bulk milk

Bulk milk samples were a part of the protocol for each sampling; however, they were for unknown reasons not obtained in 10/43 (23%) of the sampling events (Additional file 2). Furthermore, six samples were lost after the initial bacteriological analysis and, therefore, not

Table 3 Median (interquartile range) of somatic cell count (× 1000 cells/mL) according to *Staphylococcus aureus* status[a], parity and sampling period

Parity	Sampling period							
	Indoor/early lactation		Pasture/mid lactation		Indoor/late lactation		n samples (n goats)	
	S.aur0	S.aur1	S.aur0	S.aur1	S.aur0	S.aur1	S.aur0	S.aur1
First	100 (50–223)	715 (163–2720)	270 (110–690)	1395 (460–3083)	230 (90–545)	1650 (355–2869)	888 (177)	33 (22)
Second	150 (70–410)	1530 (550–2950)	690 (265–1285)	4752 (2003–6831)	410 (170–950)	1756 (1099–2115)	906 (168)	79 (38)
Third	170 (70–400)	2265 (1205–3373)	680 (278–1452)	6450 (2602–9923)	445 (140–930)	4496 (-)[b]	471 (103)	19 (9)
Fourth	170 (70–423)	1510 (1023–1905)	1270 (420–2608)	6927 (3790–9989)	530 (240–1005)	2155 (1628–4555)	525 (114)	45 (21)
≥ Fifth	190 (90–483)	2130 (980–2540)	1190 (698–2420)	3640 (1370–4490)	585 (280–1213)	2265 (1880–3281)	848 (191)	34 (18)

[a] *Staphylococcus aureus* detected (S.aur1) *vs*. not detected (S.aur0) in udder half milk samples analyzed by bacterial culture

[b] One observation/one goat only

Table 4 Prevalence (percent in brackets) and *spa* types of *Staphylococcus aureus* in milk samples from nine Norwegian dairy goat farms (A-I)

Sampling	Farm								
	A	B	C	D	E	F	G	H	I
1	5/83 (6)	5/61 (8)	1/107 (1)	3/66 (5)	2/63 (3)	2/82 (2)	Nd[a]	3/178 (2)	7/65 (11)
2	9/94 (10)	15/85 (18)	0/110 (0)	2/53 (4)	2/73 (3)	0/81 (0)	16/92 (17)	3/192 (2)	5/54 (9)
3	5/81 (6)	32/98 (33)	0/111 (0)	4/63 (6)	0/57 (0)	0/86 (0)	14/89 (16)	4/189 (2)	5/51 (10)
4	3/90 (3)	21/95 (22)	0/109 (0)	3/60 (5)	2/68 (3)	0/82 (0)	16/87 (18)	0/185 (0)	1/42 (2)
5	3/90 (3)	14/95 (15)	0/100 (0)	Nd[a]	1/73 (1)	0/82 (0)	20/91 (22)	0/182 (0)	2/44 (4)
Staphylococcus aureus prevalence									
Average %[b]	6	19	< 1	5	2	< 1	18	1	7
Period prevalence (%)[c]	10/97 (10)	42/104 (40)	1/113 (< 1)	5/67 (7)	4/75 (5)	2/88 (2)	26/103 (25)	8/199 (4)	11/66 (17)
n isolates *spa* typed	8	13	1	3	0	2	8	4	5
Spa types (n isolates per *spa* type)	t2678 (8)	t2678 (4) t7298 (1) t21448 (8)	t3768 (1)	t2678 (3)		t6293 (1) t8842 (1)	t586 (6) t8402 (1) t2678 (1)	t843 (3) new[d](1)	t1171 (4) t16282 (1)

[a] No sampling done

[b] Average prevalence in the five samplings

[c] Proportion of goats with *S. aureus* in at least one sampling event during the study period

[d] New *spa* type (03–16-12–21-17–23-13–17-17–17-17–17–23–31-24)

analysed by qPCR, resulting in altogether 27 bulk milk samples analysed by both bacterial culture and qPCR. Examples of bulk milk samples with complete results are shown in Table 6, and all results are presented in Additional file 2.

S. aureus was detected simultaneously by culture and qPCR in 18/27 bulk milk samples and not detected by either method in 5/27 samples. In two samples, *S. aureus* was detected only by qPCR, while in another two samples, it was positive only by bacterial culture (see Additional file 2). In 11 sampling events, the prevalence of *S. aureus* in goat-level milk samples (aggregated to herd level) was 0 (Table 4). For six of these events, bulk milk samples were analysed using both culture and qPCR. In four of these, *S. aureus* was not detected by either method, while it was detected in the two remaining bulk milk samples. Bulk milk samples from sampling events where the herd prevalence of *S. aureus* was 10 percent or higher (in goat samples aggregated to herd level) always had a cycle threshold (Ct) lower than 30, indicating a high amount of DNA (Table 6). In some samples with TBC higher than

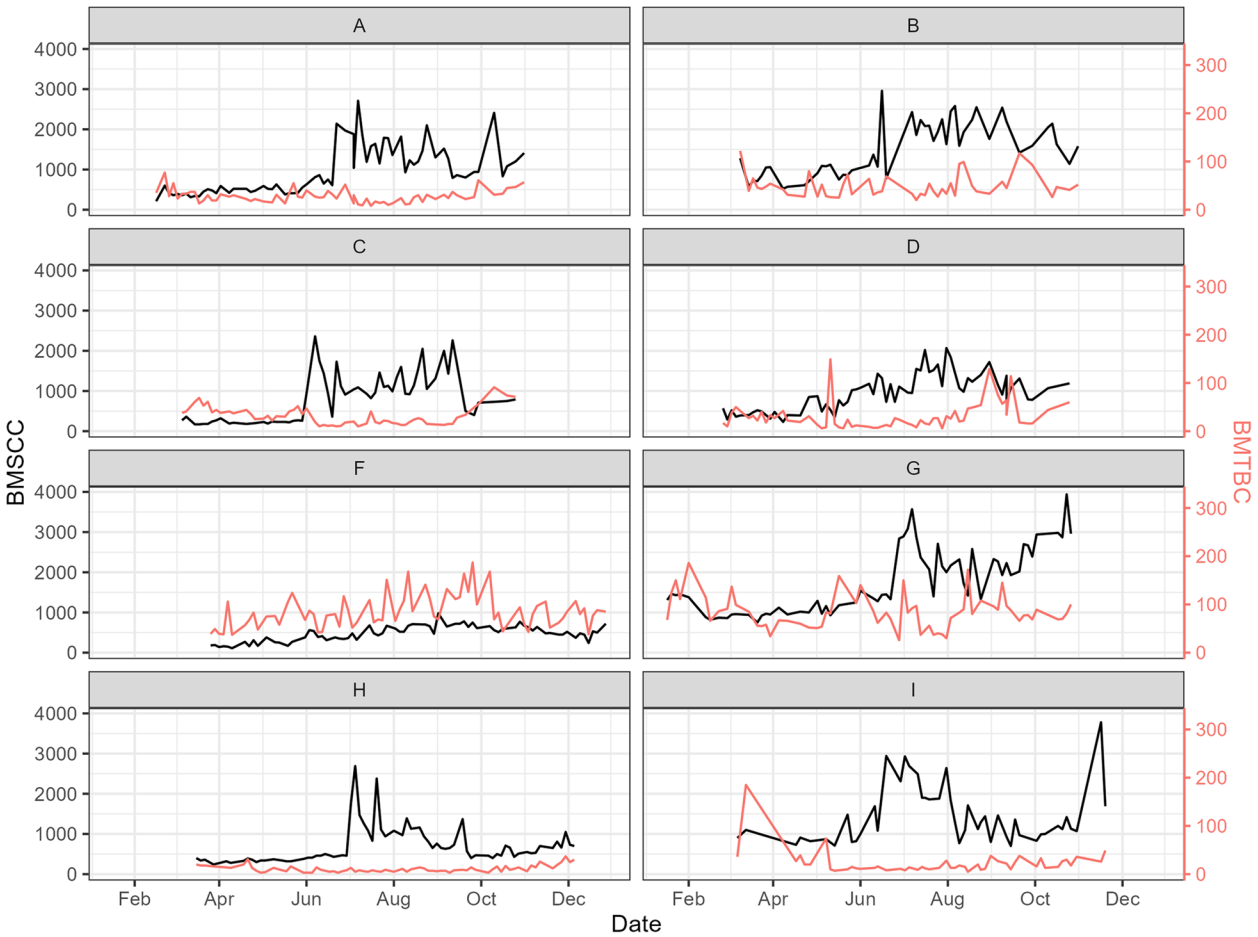

Fig. 2 Bulk milk somatic cell count (BMSCC, black line) and total bacterial count (BMTBC, red line) in 1000 cells/mL in eight Norwegian dairy goat herds during one lactation

Table 5 Description of mean (standard deviation) bulk milk somatic cell count (BMSCC) and bulk milk total bacterial count (BMTBC) (× 1000 cells/mL) in eight Norwegian dairy goat herds in 2022

	Farm							
	A	**B**	**C**	**D**	**F**	**G**	**H**	**I**
BMSCC								
Indoor/early lactation	492 (143)	876 (221)	254 (142)	656 (312)	236 (108)	1111 (244)	370 (71)	1070 (403)
Pasture/mid lactation	1509 (466)	2012 (452)	1274 (456)	1365 (364)	525 (168)	2348 (723)	1127 (524)	1934 (715)
Indoor/late lactation	1188 (470)	1621 (333)	666 (128)	1138 (259)	591 (152)	2802 (531)	590 (159)	1229 (555)
Mean (all observations)	966 (587	1463 (623)	804 (577)	980 (461)	481 (205)	1713 (846)	667 (442)	1395 (673)
12-month geometric mean	806	1326	598	864	437	1535	571	1272
BMTBC								
Indoor/early lactation	30 (13)	141 (335)	41 (12)	24 (25)	67 (27)	134 (178)	11 (7)	102 (203)
Pasture/mid lactation	21 (11)	82 (136)	17 (7)	46 (45)	92 (43)	88 (47)	8 (3)	15 (8)
Indoor/late lactation	40 (14)	67 (38)	63 (20)	31 (20)	114 (105)	78 (11)	16 (9)	26 (11)
Mean (all observations)	28 (14)	106 (239)	19 (32)	33 (35)	96 (76)	111 (134)	11 (7)	47 (122)

Table 6 Results of analyses of bacteriological composition in selected[a] bulk milk samples from nine Norwegian dairy goat herds

		Method – bacteriological composition			Routine analysis	
		Bacterial culture		qPCR	TBC[b]	SCC[b]
Farm	Sam-pling	Growth	Bacteria identified by MALDI-ToF	Result (Ct)[c]		
A	2	Moderate	Dominated by *S. aureus*. Other findings: *S. caprae*/other NAS	*S. aureus* (28.3), NAS (29.0)	13	712
A	4	Moderate	*S. warneri, S. epidermidis, S. aureus,* Pseudomonas sp, Solibacillus sp	*S. aureus* (33.7), NAS (29.9), *Pseudomonas* sp (28.3)	35	1378
B	2	Rich	Mixed growth with *S. aureus, S. caprae, S. epidermidis, S. simulans*, lactococci, enterococci, Pseudomonas sp	*S. aureus* (27.7), NAS (26.3), *Enterococcus* (29.5), *Pseudomonas* (18.3), *Enterobacter* (28.3)	450	1114
B	3	Rich	Dominated by Pseudomonas sp. Other findings: *S. warneri, Str. dysgalactiae, S.aureus*	*S. aureus* (27.4), NAS (28.3), *Str. dysgalactiae* (23.5), *Enterococcus* (28.5), *Pseudomonas* (17.9)	2806	2550
C	2	Not evaluated	Mixed growth dominated by Kocuria sp. Other findings: *Enterococcus faecalis, Chrysebacterium, Lactococcus sp, S. warneri*	*S. aureus* (not detected), NAS (29.1), *Enterococcus* (32.0), *Pseudomonas* (30.5)	56	224
D	2	Moderate	Mixed growth, *S. aureus, S. caprae, Enterococcus faecalis*, Pseudomonas sp	*S. aureus* (32.1), *Pseudomonas* (25.1)	11	944
D	3	Not evaluated	Mixed growth. *S. aureus, S. warneri, Enterocuccus durans, Lactococus lactis*	*S. aureus (29.9),* NAS *(30.8), Pseudomonas (22.4)*	148	1005
F	2	Moderate	Mixed growth dominated by *S. warneri* and *S. caprae*	*S. aureus* (not detected), NAS (30.1), Pseudomonas (26.0)	70	333
G	3	Rich	Rich growth of *S. aureus*	*S. aureus* (26.0), *Enterococcus* (30.1)	125	1412
G	4	Rich	Rich growth of *S. aureus*. In addition, growth of *S. caprae*	*S. aureus* (25.5), NAS (26.3), *Pseudomonas* (27.2)	124	2102
H	2	Moderate	Mixed growth, *S. aureus, S. chromogenes, S. epidermidis, S. warneri, Enterococcus faecalis*	*S. aureus* (34.3), NAS (30.4)	13	375
H	3	Sparse	*S. aureus, S. epidermidis, S. caprae, Lactococcus lactis*	*S. aureus* (33.6)	8	1056
I	1	Rich	Rich growth of *S. aureus*	*S. aureus* (22.8), NAS (30.5)	Not done	Not done
I	3	Sparse	*S. epidermidis, S. pasteuri,* Corynebacterium sp and *S. aureus*	*S. aureus* (32.9)	12	3135

[a] The complete set of bulk milk analyses is provided in Additional file 2. *Staphylococcus* is abbreviated *"S."* and *Streptococcus* is abbreviated "*Str*". Non-aureus staphylococci are abbreviated "NAS"

[b] TBC = Total bacterial count, SCC = somatic cell count in 1000 cells/mL in bulk milk as measured in the routine analyses

[c] qPCR results other than *S. aureus* are reported for targets with cycle threshold (Ct) < 32 only

100.00 cells/mL, the Ct-value for *Pseudomonas* sp was very low (< 20), but the dominance of *Pseudomonas* sp was not always seen by culture (Table 6/Additional file 2).

Statistical models

Data were combined into three categories for parity: i) first parity, ii) second and third parity, iii) fourth parity or higher, as well as stratified by season in goats of parity four or higher, giving a total of four different statistical models defining adjusted SCC thresholds (Table 7). The SCC cutoffs that were significant for *S. aureus* detection differed between parities, where a first parity goat would need attention already at a SCC of > 500.000 cells/mL, whilst the threshold was > 3,000,000 cells/mL for a goat of fourth parity or higher on pasture (Table 7). Milk yield and lactation stage were tested as covariates in the models to adjust for the effect of these physiological parameters on goat SCC but was only retained in the model including first parity goats.

Discussion

This study focused on the interpretation of SCC and other diagnostic tests with potential value in the udder health work in dairy goat herds. *S. aureus* was confirmed as the udder pathogen with the strongest influence on SCC. Identifying and controlling subclinical mastitis caused by *S. aureus* is essential to improve milk quality and prevent clinical mastitis in dairy goats. As a first step towards designing udder health monitoring programs for goats, the paper explored adjusted SCC thresholds for the detection of *S. aureus* at the goat level and the use

Table 7 Odds ratio (OR) for different somatic cell count (SCC) cutoffs in four mixed logistic models stratified by parity, with *Staphylococcus aureus* 0/1 as outcome, and SCC (×1000 cells/mL) as independent variable

Model 1:		**Model 2:**		**Model 3:**		**Model 4:**	
Parity 1		**Parity 2 and 3**		**Parity ≥ 4 (indoor)**		**Parity ≥ 4 pasture**	
SCC category	**OR (95% CI)**	**SCC category**	**OR (95% CI)**	**SCC category**		**SCC category**	**OR (95% CI)**
0–500	Referent	0–1500	Referent	0–1500	Referent	0–3000	Referent
500–1000	6.1 (6.09 – 6.11)***	1500–2000	4.7 (0.84–26.6)*	1500–3000	6.57 (1.11–38.90)**	> 3000	12.2 (3.84 – 38.69)***
1000–1500	23.7 (23.6 – 23.7)***	> 2000	8.4 (2.53–27.7)***	> 3000	16.70 (1.42–196.4)**		
> 1500	140.5 (140.2 – 140.8)***						
N cases	79		98		53		26
N controls	900		1371		1080		284
AUC[a]	0.99		0.99		0.99		0.95
AUC[b]	0.80		0.77		0.78		0.73

Significance level: *$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

[a] Area under the ROC-curve (AUC), model with random effect goat nested within herd included

[b] AUC in model without random effect included

of bulk milk testing for the identification of udder health problems caused by *S. aureus* at the herd level.

Milk recordings are necessary to monitor and identify goats that cause problems. In this study, five milk recordings were performed during one lactation, which provided a relatively good basis for identifying goats suspicious of having an IMI. The levels of SCC in goats with *S. aureus* were, in most cases, well distinguishable from goats that were culture negative or with NASM when different parities and lactation stages were investigated separately. Hence, the study shows that SCC is a valuable screening tool for *S. aureus* when adjusting for other known sources of elevated SCC. Due to the large variation in SCC of non-infectious origin in goats, it is not appropriate to have one common cutoff to define a goat udder as healthy or diseased across different parities and lactation stages. This was also demonstrated in [22], where the best balance between sensitivity and specificity of SCC for detecting goats with *S. aureus* in late lactation was found at 2 million cells/mL, but it had a relatively low accuracy (sensitivity: 78.2%, specificity: 61.5%).

The recommended threshold for submitting milk samples for bacteriological culture in Norway is 1 million cells/mL, preferably based on the mean of two or three milk recordings. The results of this study show that this threshold should be set lower in first parity goats in early lactation and higher for goats of higher parity and later lactation. The interquartile ranges of SCC were more overlapping in goats with and without *S. aureus* identified during pasture, especially in goats of higher parities. This underlines the challenge of identifying goats with IMI during the pasture season. Although adjusted SCC thresholds showed promising results for differentiating "healthy vs unhealthy" udders in this study, confirmation of IMI with bacterial identification is still necessary.

According to IDF (2022), confirmation of ≥ 100 cfu/mL is sufficient for diagnosing a *S. aureus* IMI under practical conditions [15]. Normally, the animals will not be sampled if their SCC is low, but in this study, we sampled all goats at each farm visit. Goats with *S. aureus* that had a SCC < 1 million cells/mL were more likely to have few *S. aureus* colonies (< 500 cfu/mL), and to be negative at the next sampling event. Some of these *S. aureus* findings are therefore likely to be contamination or teat canal colonisations, and not true IMI. We may, therefore, have overestimated the prevalence of *S. aureus* IMI in this study. The findings underline that results with few *S. aureus* and a low SCC should be interpreted with care. Rather than culling the goat after such findings, a follow-up bacteriological sampling can be done if the goat has high SCC at the next milk recording.

Due to the relatively long sampling interval, the infection dynamics of *S. aureus* could not be strictly evaluated. However, based on the five samplings, the spread of *S. aureus* from one sampling event to the next seemed to differ between the herds. To investigate whether specific genotypes of *S. aureus* were associated with high within-herd prevalence, we added *spa* typing of selected *S. aureus*-isolates. We found a relatively high diversity with 12 detected *spa*-types, with one to three *spa* types per herd. A previous investigation in seven Norwegian dairy goat herds also found a relatively high diversity of *S. aureus* using pulse-field gel electrophoresis [30].

The most frequently identified *spa* type, t2678 belongs to clonal complex 133, which is the dominant lineage in sheep and goats [23, 31]. Other *spa* types with high within-herd prevalence in this study included t586 and t21448. Since none of the goats had clinical mastitis at the time of sampling, all *spa* types identified were associated with subclinical mastitis.

Previously, we identified a herd-specific pattern of bulk milk SCC, which was similar from year to year [26]. The same research group has also shown in a study of bulk milk from 88 Norwegian dairy goat herds that the variability in the bulk milk SCC is high, particularly during the pasture season [32]. Although previous investigations point towards mainly non-infectious sources of variability in goat bulk milk somatic cell count, we identified some patterns in bulk milk SCC and TBC in this study that could indicate an udder health issue caused by *S. aureus*. A high level of bulk milk SCC accompanied by higher and more unstable bulk milk TBC should increase awareness of udder health. To define "high levels" of bulk milk SCC, the lactation stage and season must be considered [32]. The three herds with the highest prevalence of *S. aureus* (farms B, G, I) also had the highest levels of bulk milk SCC. These herds had a 12-month geometric mean SCC of more than 1,2 million cells/mL, which has been the cutoff for premium payment for Norwegian goat milk. Notably, also in this study, the bulk milk SCC varied greatly between the herds, not only due to differences in udder health. The level of bulk milk SCC in dairy goats is influenced by the distribution of parities in the herd, the management system, and whether the herd is on pasture [27, 32]. Possibly, factors related to stress are also influencing the bulk milk SCC [33, 34], including the stocking density, the group size, the use of concentrate feeders, environmental enrichments, the bulk space, and the availability of forage. The included herds varied a lot with respect to these possible stressors; however, investigating the impact of stress was beyond the scope of this paper. Furthermore, the period on mountain pasture probably involves even more differences related to animal stress, feed intake and movement. With this large variability of factors of non-infectious origin, it is more relevant to define the reaction thresholds for bulk milk SCC in pasture-based management systems on historical data from the same farm than to benchmark with other farms. Further, more investigations are needed regarding the connection between stress and SCC in dairy goats.

Bulk milk bacterial diagnostics provided additional information to the routine measurements. *S. aureus* was identified by both bacterial culture and qPCR, even at low herd prevalence. The identification of *S. aureus* in bulk milk in herds with an apparent herd prevalence of zero is not surprising given the many extramammary sources of *S. aureus* in dairy goats [30, 35]. In sampling events where a *S. aureus* prevalence higher than 10% was found in goat samples, the Ct-values of qPCR results from bulk milk samples were usually below 30 (Table 6). This suggests that qPCR analyses may be a relatively good indicator of a *S. aureus* problem in a herd. However, the sample size was small, and this finding needs further validation. Dominance of *S. aureus* in bulk milk samples was associated with a moderately increased TBC, but the results indicate that *S. aureus* IMI may cause levels exceeding 100,000 bacterial cells/mL, which has been the cutoff for premium payment related to TBC for both cow and goat milk in the Norwegian milk production.

Some bacterial findings associated with milk quality issues in dairy cows were identified in the bacterial analyses of the bulk milk samples: *Pseudomonas* sp was identified by qPCR with low Ct-values in several samples with high TBC (Table 5). *Pseudomonas* sp are psychrotrophic bacteria commonly found in bulk tank milk and has been associated with environmental contaminations at the farm and is a low-incidence cause of mastitis in Norway [36]. Despite low *Pseudomonas* sp Ct-values in the sample, the bacterial group rarely dominated by the culturing method. The culturing method used in this study was the standard conditions used for bacterial culture for identification of udder pathogens, with blood agar and incubation at 37 °C. For improved detection of psychrotrophic bacteria like *Pseudomonas* sp, other culturing media and culturing conditions are required [16].

Farm F had an elevated TBC from routine bulk milk samples compared to the other herds during the whole study period, whilst the bulk milk SCC was the lowest of all herds. Unfortunately, many of the bulk milk samples for bacteriology from this farm were lacking, and the analyzed samples had a TBC of less than 100.000 bacterial cells/mL. The source of the high TBC in this farm could therefore not be identified. However, due to the good udder health in the herd (low *S. aureus* prevalence, and NASM prevalence comparable with the other farms), we considered the most likely source to be issues with the cleaning of the milking machine or biofilm formation. The focus of this study was the identification of udder health problems, and methods for identification of bacterial groups contributing to high TBC originating from sources other than IMI should be further investigated.

The qPCR-kit utilized in this study identified several bacterial groups that dominated in samples with elevated TBC in goat milk, including *S. aureus, Str. dysgalactiae* and *Pseudomonas* sp. Combining a quantitative test like TBC and qualitative tests like culturing or

qPCR can give valuable information to solve problems in farms with milk quality issues faster. The interpretation of the results can be challenging, and repeated sampling can improve the usefulness of bulk milk samples, as also recommended by [37].

Conclusions

This study provides new knowledge on how to combine routinely measured parameters with information obtained from laboratory tests on individual and herd level in dairy goats, which is the first step to design efficient udder health monitoring programs adapted for goat milk production. To identify udder health problems in dairy goats, the farmers and advisors need to differentiate elevated SCC caused by intramammary infections from non-infectious causes. Cutoffs that were significant for *S. aureus* IMI ranged from 500,000 cells/mL in first parity goats in early lactation to 3 million cells/mL in older goats on pasture. The patterns of routinely measured bulk milk parameters, combined with the testing of bacterial composition, demonstrated a promising approach for identifying herds with udder health issues.

Methods

Study design

Nine dairy goat farms (A-I) were visited by advisors or researchers in the project group five times between March and November 2022. Farms A-D were also described in the first part of this study, where we investigated factors influencing goat SCC during two lactations [26]. The herds were sampled during three seasons, which coincide with the lactation stage in Norway: Sampling one and two during indoor feeding/early lactation, sampling three during pasture/mid-lactation, and sampling four and five during indoor feeding/late lactation. The included goat herds had kidding season from February to April, giving an approximate lactation stage of the herd of less than 100 days in milk for sampling one and two (indoor early lactation), 100–200 days in milk for sampling three (pasture mid-lactation) and > 200 days for sampling four and five (indoor late lactation).

We selected the farms based on geographic regions to include the three geographic regions with goat milk production in Norway: five farms (A-E) from eastern Norway, two farms from western Norway (F, G) and two farms from northern Norway (H, I). Furthermore, we aimed to include farms with suspected good udder health and farms with some udder health challenges. This inclusion criterium was based on the researchers' and advisors' expertise evaluating the levels of bulk milk SCC the year before the study, where a geometric mean of all deliveries of more than approximately 1,2 million cells/mL was considered high (suspected udder health challenges, farms B, G, I), and less than approximately 800,000 cells/mL was considered low (suspected good udder health, farms A, C, D, F, H). Six of the herds (farms A, C, D, E, F, G) were moved to a mountain farm during the summer, either by transport (A, D, E, G) or walked (C, F).

Sampling

At each farm visit, udder half milk samples for bacteriological analysis were collected from all lactating goats prior to milking. The initial streams of milk were discarded, the teat was disinfected with a cotton swab soaked in 70% alcohol, and 5 mL of milk was then collected into a sterile tube from each udder half. The farmer or advisor collected milk recording samples with milk meters from all goats in connection to the sampling. For practicality and time restraint reasons, the milk recording samples were obtained within one day before or after the bacteriology sampling and both on morning and evening milkings. Bulk milk samples (100 mL) were collected from the top of the bulk tank using sterile sampling equipment designed for hygienic sampling of milk (Bulkotest, Skala AS, Oslo, Norway) and transferring 5 mL milk to a sterile tube after the milk in the tank was stirred. In addition, results from bulk milk analyses (SCC, TBC) from the routine analyses from each milk collection (collected every third day) during the whole study period were retrieved from the dairy company, TINE. The samples are collected automatically during the milk transfer from the bulk tank to the milk truck ensuring a representative bulk milk sample. Farm E did not deliver milk to TINE, and routine bulk milk analyses were therefore not available.

Analyses of samples

An overview of the analyses of the samples collected during the study period is provided in Table 1, and the details regarding analyses are provided in the following sections.

Goat milk samples

The milk recording samples were conserved with bronopol (sampling 1, 5) or transported cooled (sampling 2–4) to the laboratory and analyzed for SCC by Bentley FTS/FCM (Bentley Instrument Inc, Chaska, MN, USA).

Udder half milk samples were transported cooled, and frozen (−20 °C) upon arrival at the laboratory. The milk samples were thawed and analyzed using bacterial culture according to standard accredited procedures of the TINE Mastitis laboratory [16, 36]. Briefly, a milk volume of 0.01 mL from each udder half was spread on washed 5% cattle blood agar plates with esculin and incubated at 37 °C. Plates were read at 24 h and 48 h. *S. aureus* was

identified by typical colony morphology and a betatoxic haemolysis zone, or with MALDI-ToF (Bruker Daltonics, Bremen, Germany). *S. aureus* was reported as "*S. aureus*" if there were more than five colonies (≥500 colony forming units [cfu]/mL), or "*S. aureus*, sparse growth" if one to five colonies (100–500 cfu/mL). All other colonies were identified with MALDI-ToF if they grew in pure culture and with ≥500 cfu/mL. Samples yielding more than two colony types were reported as contaminated and excluded from the statistical analyses.

A selection of *S. aureus* isolates was subtyped using *spa* typing [30]. Depending on the available isolates, 1–13 isolates per farm were selected, except for farm F, where no isolates were available. One isolate per goat was selected, except for one udder half sample with *S. aureus* with different colony morphology, where both colony types were confirmed as *S. aureus* by MALDI-ToF and selected for *spa* typing. Amplification of region X of the *spa* (protein A) gene was performed using primers spa-1113f (5′-AAA GACGATCCTTCGGTGAGC-3′) and spa-1514r (5′-CAGCAGTAGTGCCGTTTGCTT-3′) [31]. PCR amplification was performed using Roche LightCycler 480 with the following protocol: 95 °C for 30 s, 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 68 °C for 70 s and lastly 68 °C for five minutes.

The PCR product was purified using Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, CA), according to the manufacturer's instructions, and was submitted to GATC Biotech AG (Konstanz, Germany) for sanger sequencing. Sequences were processed using Geneious V7 software, and the *spa* types were determined using spaTyper tool, version 0.3.3 (https://github.com/HCGB-IGTP/spaTyper).

Bulk milk samples

The dairy company analyzed SCC, TBC, and chemical components from samples collected at each milk collection. Somatic cell count was measured by Bentley FTS/FCM (Bentley Instrument Inc), and TBC by BactoCount IBC (Bentley Instrument Inc).

Bulk milk samples from the 5 samplings were analysed by bacterial culture and qPCR. Bacterial culture was performed by plating 0.1 and 0.01 mL on washed 5% cattle blood agar plates with esculin and read at 24 and 48 h. In analysis of bulk milk samples, different volumes are plated to be able to identify colony types with MALDI-ToF in samples with high bacterial load. The 2–4 dominating colony types were identified with MALDI-ToF.

The qPCR (Mastit 4 M4BDFT, DNA Diagnostic, Denmark) included the 16 targets *S. aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Mycoplasma bovis*, *Mycoplasma* sp, *Klebsiella* sp, the BlaZ gene (staphylococcal betalactamase gene), Non-aureus staphylococci (NAS), *Prototheca* sp, *Escherichia coli*, *Pseudomona*s sp, *Streptococcocus* sp, *Enterococcus* sp/*Lactococcus lactis* ssp. *lactis,* and *Bacillus/Clostridium* sp. The DNA extraction and qPCR testing were performed according to the manufacture's protocol (DNA Diagnostic, 2020), with a volume of 500 µl milk. Plate Washer (BioRad DW40) and Multifuge X4R PRO (ThermoFisher Scientific) were used for DNA extraction. The real-time PCR instrument CFX96 Touch Real-Time system (BioRad) was used for amplification. According to the manufacturer's instructions, a cycle threshold of <37 was considered positive for the target. However, with the focus on *S. aureus* in this study, as well as other bacteria with a significant presence in bulk milk, it was decided to report other targets than *S. aureus* at a cycle threshold <32 based on [38].

Statistical analyses

Data were analyzed in R, version 4.1.3 (R Core Team, 2023). Observations lacking results from either bacteriological culture or milk recordings were excluded from the descriptive statistics. Results from bacteriological culture were first summarized at the udder half level. Since SCC was measured in composite goat samples, udder half bacteriology results were aggregated to a goat-level bacterial status for the most frequently detected udder pathogens: Samples with *S. aureus* detected in one or both udder halves were classified as "*S. aureus*". Samples with other common bacterial findings were classified as that bacterium in the descriptive statistics if they had only this finding (one or both udder halves). Contaminated samples were excluded from further statistical analyses. Since the distribution of SCC is right skewed, we used the ln-transformed SCC in the descriptive figures.

A dichotomous variable, *S. aureus* status, was generated for *S. aureus* (0/1, *S. aureus* not detected *vs* detected). The SCC was summarized by *S. aureus* status, parity and season. Based on the distribution of SCC, an ordinal variable (SCC class) was generated, with SCC cutoffs 200, 500, 1000, 1500, 2000, 3000 (×1000 cells/mL). Initially, the distribution of *S. aureus* positive and negative goats was summarized according to parity and SCC classes. SCC classes or parity classes were combined if necessary to include a sufficient number (>3) of *S. aureus* positive goats across the different sampling periods.

The prevalence of *S. aureus* positive goats was calculated for each sampling event, and the period prevalence was calculated for the study period. The period prevalence was calculated as the proportion of goats positive for *S. aureus* in at least one sampling event during the study period.

Mixed effects logistic models were specified on datasets stratified by parity (first parity, second and third

parity, parity fourth or higher) to identify the best cutoff for *S. aureus* detection based on SCC-classes. For parities fourth or higher, the dataset was also stratified into indoor season and pasture because older goats have a stronger SCC response on pasture [26]. *S. aureus* (1/0) was the outcome and SCC class was the independent variable. Milk yield and season influence the SCC and were therefore tested as covariates in the models and retained if the significance level was $p < 0.05$. Goat nested in herd was included as random effect when repeated measures per goat was included. To choose between competing models the Akaike information criterion (AIC) and the Bayes information criterion were used. Model assumptions and quality of the model fit was evaluated by visual inspection of the residual plots. The initial model tested within each parity was as follows:

$$\ln\left(p_{ij}/\left(1-p_{ij}\right)\right) = \mu + SCC\_CLASS_{ij} + SEASON_{ij} + MY_{ij} + \sigma_{ij} + \epsilon_{ij}$$

where p is the probability that *S. aureus* was detected in the udder of the ith goat in the jth herd, μ represents the intercept, SCC_CLASS represents the different SCC-classes described above, SEASON represents the sampling period (indoor/early lactation, pasture/mid lactation, indoor/late lactation), MY represents the milk yield in kg milk at the test day, σ_{ij} represents the repeated variation of the ith goat within the jth herd, and ϵ_{ij} represents the residual error.

The models were first run with the initial SCC class with cutoffs described above. Cutoffs that had a non-significant association with *S. aureus* were then combined with the higher cutoff, and the models were run until the SCC-cutoffs were significant ($p < 0.05$). Hence, the final models identified the SCC cutoffs (corrected for the milk yield and season, if significant) with the odds ratio for having *S. aureus* at the given SCC level.

We included milk yield in the models to correct for the known dilution effect on SCC [39, 40]. One farm (G) had missing values for milk yield at two samplings because milk meters were not available at the mountain farm. Milk recording samples were therefore obtained manually, and milk yield was not measured. In total, 256 recordings of milk yield were missing, corresponding to seven percent of all milk yield recordings, which is considered low missingness [41]. Based on our knowledge of farm G and the farmer, we had no reason to assume that the missing milk recordings were intentional or related to the actual milk yield at the mountain farm. Therefore, the missing yield recordings could be considered missing at random (MAR). Given that the data are MAR, using a multiple imputation is preferred over simpler methods like e.g. mean imputation, which underestimates the variance and bias the estimate of the mean when data are not missing completely at random (MCAR) [42]. Similarly, listwise deletion, another commonly used method, produces an estimate of the standard error that is too large, and produces a biased estimate of the mean when data are not MCAR, an assumption often unrealistic for the data at hand [42]. Multiple imputation creates several complete versions of the data by replacing the missing values by plausible data values based on the remaining information in the dataset. These plausible values are drawn from a distribution specifically modeled for each missing entry. The results are pooled into a final point estimate plus standard error by pooling rules ("Rubin's rules") [42].

In this study, the missing values were imputed using multiple imputation by chained equations implemented in the MICE package in R, version 3.15.0 [43]. In MICE each variable has its' own imputation model, and MICE fills in missing data in the dataset through an iterative series of predictive models. The MICE algorithm requires a specification of a univariate imputation method separately for each incomplete variable. Imputation methods tested were predictive mean matching, classification and regression trees, lasso linear regression and random forest. The distribution including imputed values was compared with the original distribution. Based on comparison of the distributions and the author's earlier experience with the method, random forest was chosen. Except for the choice of imputation method, the default settings in MICE were used.

The area under the ROC-curve (AUC) and odds ratios were calculated for each model. For comparison of AUC, the same models were also specified without the random effects.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04556-8.

Additional file 1: Figure A1. Prevalence (percent infected udder halves) of the most common bacteriology findings at each farm during five sampling events. Table A1. Somatic cell count (×1000 cells/mL), median, quartile 1 (q1) and quartile 3 (q3) according to S. aureus status (*S. aureus* detected SAUR = 1, *S. aureus* not detected SAUR = 0), parity and sampling period. Observations with sparse growth of *S. aureus* (1–5 colony forming units) were excluded. Number of observations (n) and number of goats (n_goats).

Additional file 2. Results of analyses of bacteriological composition in bulk milk samples from nine Norwegian dairy goat herds.

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Authors' contributions
Conceptualization was performed by MS and RAI. Methodology was performed by MS, MKV, DP and BGH. Validation was performed by MS, MKV, DP. Formal analysis was performed by MS, MKV, FVFA. Writing of the original draft was performed by MS. Review and editing of the manuscript were performed by all authors. Project administration was performed by SS and RAI. Last, funding acquisition was acquired by SS and RAI. All authors have read and approved the final version of the manuscript.

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Data availability
The data generated and analyzed in this study have been used by the permission of TINE and MIMIRO who own the data. The data might be available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
The sampling performed in this observational study did not require ethical approval. The farmers provided permission for the sampling and for the use of their information in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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