Estimating test characteristics of somatic cell count to detect *Staphylococcus aureus*-infected dairy goats using latent class analysis

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ABSTRACT

The aim of this study was to estimate test properties of composite somatic cell count (cSCC) to detect subclinically *Staphylococcus aureus*-infected dairy goats. *Staphylococcus aureus* is the most prevalent major pathogen in goats, and responsible for the majority of clinical mastitis cases. Therefore, a diagnostic tool that detects subclinical *Staph. aureus* infections may be useful in decreasing the number of clinical cases. We collected samples from 384 animals in 4 herds for bacteriological culture and cSCC on 3 occasions in lactation: once in early lactation, once around peak lactation, and once in late lactation. Latent class models were used to estimate test properties of cSCC and bacteriological culture in the absence of a gold standard reference test under the assumption that both tests detect *Staph. aureus* intramammary infection. Estimates for test properties of cSCC in early lactation at a cut-off value of $1,500 \times 10^3$ cells/mL were 0.90 for sensitivity and 0.95 for specificity, making cSCC a useful screening tool for detection of *Staph. aureus*. An effect of lactation stage was observed, causing an increased sensitivity and decreased specificity in late lactation. The sensitivity of bacteriological culture was estimated to be very low in the latent class models and the models suggested that the true prevalence of *Staph. aureus* in dairy goat herds is much higher than what is commonly reported based on bacteriological culture. This implies that intramammary infection by *Staph. aureus* may be an underestimated problem in dairy goat herds, and that cSCC can be used to diagnose infected animals.

Key words: somatic cell count, latent class, test characteristic, dairy goat

INTRODUCTION

Somatic cell count is frequently used to detect udder health problems in dairy animals. Especially in dairy cattle, it is widely used in regulatory systems

and available throughout the dairy industry in the world (Schukken et al., 2003). But also in the dairy goat industry, SCC is frequently used to detect udder health problems. Most intramammary infections in goats are caused by staphylococci (Contreras et al., 2003). Coagulase-negative staphylococci are usually associated with subclinical infections. *Staphylococcus aureus* can also be cultured from subclinically infected goats, but is the most frequent cause of clinical mastitis. Because of the severity of clinical symptoms associated with mastitis caused by *Staph. aureus*, and because of the capacity of this organism to produce thermostable toxins, eliminating *Staph. aureus*-infected animals from the herd is important (Contreras et al., 2007). To identify these animals in a subclinical stage of infection, SCC may be a useful screening tool. It has, however, been shown that not only IMI, but also several noninfectious factors, such as parity and stage of lactation, affect SCC in goats (Luengo et al., 2004; Min et al., 2007; Paape et al., 2007). Therefore, some authors have questioned the usability of SCC in goat milk. Nevertheless, several studies have estimated test characteristics of SCC for detecting IMI (Table 1) and found results comparable to or slightly worse than what has been reported for cattle by Schepers et al. (1997), who reported a sensitivity (**Se**) of 0.75 and a specificity (**Sp**) of 0.90 at a threshold of 200,000 cells/mL relative to bacteriological culture.

The test properties described in Table 1 are calculated with bacteriological culture as reference test. Currently, bacteriological culture is seen as the best available method to identify IMI (Torres et al., 2009), but it cannot be seen as a perfect reference test (Erskine and Eberhart, 1988; Sanford et al., 2006). First, not all pathogens that are present in an infected mammary gland will be present in a milk sample. Some pathogens, especially staphylococci, are known to be shed intermittently (Sears et al., 1990) or the bacteria may be shed in too low amounts to be present in the inoculum volume (Buelow et al., 1996a; Lam et al., 1996). But even if a pathogen is present in the inoculum, it is not always detected with conventional culture methods (Sol et al., 2002). This implies that bacteriological culture is not

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perfectly sensitive test. False positive findings can also be obtained, as a result of teat canal infections, teat skin infections, or contamination. When bacteriological culture is used as a reference test, test characteristics of SCC will be biased and, therefore, be moderate, even if SCC were a perfect test. Other methods are needed to obtain unbiased estimates of SCC test characteristics in absence of a true gold standard.

Methods for estimating Se and Sp of diagnostic tests when the true disease status is unknown were reviewed by Enøe et al. (2000). One of these is the use of a latent class model as specified by Hui and Walter (1980). With latent class models one can estimate the Se and Sp of 2 or more tests together with the true disease prevalence in 2 or more populations (Hui and Walter, 1980). This model makes 3 assumptions: the tests are conditionally independent given disease status, each test has the same test characteristics across populations and the prevalence of true disease status differs between populations (Hui and Walter, 1980). The parameters of interest can be estimated by a maximum likelihood procedure (Hui and Walter, 1980) or by Bayesian inference (Branscum et al., 2005). Sanford et al. (2006) used Bayesian latent class analysis to estimate test characteristics of the California Mastitis Test in cattle. To our knowledge, the present study is the first to use latent class models to estimate test characteristics of SCC in goats. In this paper we aim to estimate test characteristics of composite SCC (cSCC) for detection of *Staph. aureus*-infected goats.

MATERIALS AND METHODS

Study Design and Data Collection

Four Dutch dairy goat herds were selected based on willingness of the farmer to cooperate, accuracy of animal identification and registration and the expected dates of kidding. On 3 farms, we selected 100 goats and from another farm 130 animals, stratified on parity: about 30% of the animals were yearling does, 30% were about to start a second lactation, and the rest were older animals. On one farm, only first and second parity goats were present so 30% first parity and 70% second parity animals were selected from this herd. Animals were selected in the milking parlor in the week before the first sampling. Based on the ratio of the farmer's estimate of the available number of animals that was in early lactation and of the required parity, and the number of animals required for the study, every nth animal entering the milking parlor was marked with a plastic leg tag. However, because the number of available animals was not always known exactly, the ratio of selected animals varied somewhat during the process.

Table 1. Literature overview of sensitivity (Se) and specificity (Sp) of SCC in dairy goats at threshold values of 500×10^3 cells/mL (SCC500) and $1,000 \times 10^3$ cells/mL (SCC1000) for detection of all pathogens or only major pathogens in udder-half level or composite milk samples

Reference	Pathogen	Level	SCC technique ¹	n	Prevalence	SCC500		SCC1000	
						Se	Sp	Se	Sp
Hall and Rycroft (2007)	All	Half	SomaScope	159	0.33	0.78	0.77	0.61	0.93
Sung et al. (1999)	All	Half	Fossomatic	209	0.43	0.78	0.51	0.63	0.71
Contreras et al. (1996)	All	Half	Fossomatic	369	0.18	0.69	0.61	0.53 ²	0.72 ²
Lerondelle et al. (1992) ³	All	Half	Fossomatic	181	0.25			0.42	0.95
Kalogridou-Vassiliadou et al. (1992)	All	Composite	Fossomatic	1,523	0.81	0.82	0.76	0.56	0.95
Kalogridou-Vassiliadou et al. (1992)	Major	Composite	Fossomatic	1,523	0.17	0.95	0.35	0.80	0.61
Lerondelle and Poutrel (1984)	Major	Half	Coulter counter	2,428	0.08			0.72 to 0.90 ⁴	
Poutrel and Lerondelle (1983)	Major	Half	Fossomatic	325	0.15			0.85	0.75

¹SomaScope (Delta Instruments, Drachten, the Netherlands); Fossomatic (Foss, Hillerød, Denmark); Coulter counter (Coulter Electronics, Margency, France).

²Threshold was 950×10^3 cells/mL in this paper.

³Viral infections were also included in this study.

⁴Estimates for Se differed with stage of lactation (respectively mid- or late lactation).

The selected animals were sampled for bacteriological culture and SCC on 3 occasions during the lactation. The first sampling took place between the end of January and early March, when the animals were in early lactation. The second was 6 wk later at around peak production and the final sampling was arranged within 2 mo after the breeding buck was introduced in the herds. This sampling moment was not the same for all herds, and varied between 24 and 36 wk after the first sample and took place between August and early October. Duplicate foremilk samples were collected from both udder halves for bacteriological culture. Samples for bacteriological culture were taken before the morning milking. Three squirts of milk were discarded and the teat-end was scrubbed with cotton soaked in alcohol. About 8 mL of milk was collected in a sterile vial. Disinfection was repeated before the second sample of the udder half was taken, and the procedure was repeated for the other half. The milk samples were cooled with ice-packs and immediately transported to the laboratory for SCC measurement (Fossomatic 5000; Foss, Hillerød, Denmark) and for conventional bacterial culture, which was done according to NMC guidelines (Hogan et al., 1999). During the third sampling occasion, a pooled sample for each goat was also cultured for *Mycoplasma bovis*. Although freezing and thawing of milk samples before culture has been shown to increase the frequency of isolation of *Staph. aureus* in cow milk (Villanueva et al., 1991; Sol et al., 2002; Artursson et al., 2010), growth of other pathogens may be decreased after freezing (Schukken et al., 1989; Amores et al., 2010). Furthermore, freezing of milk samples will impair SCC measurement (Sánchez et al., 2005). Culturing was done on fresh milk samples in this study to be able to measure SCC in the same sample that was used for culture, and because the original intent of this study was not only to detect *Staph. aureus*, but also other pathogens. A sample was considered positive if at least one cfu was cultured from a 0.01-mL inoculum. Milk samples were considered contaminated if 3 or more different types of bacteria were seen.

One or 2 d before each sampling for bacteriological culture, composite milk samples were collected as part of the regular DHI monitoring and cSCC was determined with a Fossomatic 5000 (Foss), which is a valid technique for measuring SCC in goat milk (Zeng et al., 1999).

Cases of clinical mastitis were recorded by the farmers and records from animals that had a clinical case were excluded from the analyses. Exact kidding dates were retrieved from the farmers' management systems after conclusion of the sample collection. Only animals

that were between 4 and 42 DIM at the first sampling occasion were included in the final data set.

Interpretation of Culture Results

We used serial interpretation of the duplicate samples, so if both duplicate samples yielded the same pathogen, the udder half was considered infected with that pathogen. If one of the 2 samples was contaminated or showed no growth, the udder half was considered as culture negative. When both duplicates were contaminated, the half was considered as missing. When both duplicates yielded the same 2 pathogens, it was considered to be a mixed IMI. Mixed IMI of CNS and *Corynebacterium bovis* was reported as a separate group, but mixed IMI of a minor and a major pathogen was analyzed as the major pathogen infection (e.g., a mixed infection of CNS and *Staph. aureus* was analyzed as *Staph. aureus*). We defined *Staph. aureus*, *Streptococcus dysgalactiae*, gram-negative pathogens, and esculin-positive cocci as major pathogens.

Parallel interpretation of the 2 halves was used to obtain animal-level infection status, so if any of the 2 udder halves was infected as described above, the goat was considered infected.

cSCC Cut-Off Values

Composite SCC was calculated as the average of morning and evening composite milk samples. The following cut-off values for cSCC were considered: 100, 250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, and $4,000 \times 10^3$ cells/mL.

Data Analysis

Test characteristics of cSCC and bacteriological culture were estimated for detecting *Staph. aureus*-infected goats. For comparison, we also estimated test characteristics for detecting goats infected with any major pathogen (as defined above), and test characteristics for detecting animals infected with a major or a minor pathogen.

We used the Bayesian formulation (Branscum et al., 2005) of the latent class model described by Hui and Walter (1980), which can be used to estimate Se and Sp of both tests and prevalence in each population. In our study, the 4 different herds defined the populations with different prevalences. In each of the 4 populations, the test outcomes of test 1 (T_1 , test outcome of cSCC at a certain threshold) and test 2 (T_2 , presence or absence of *Staph. aureus*) are cross-classified in 2×2 tables.

In the model, the probability (P) of observing any of the 4 possible combinations of test outcomes (T_1+T_2+ ; T_1+T_2- ; T_1-T_2+ ; T_1-T_2-) is written in terms of Se and Sp. For instance,

$$P(T_1+T_2+) = P(T_1+, T_2+ | D+)P(D+) + P(T_1+, T_2+ | D-)P(D-), \quad [1]$$

where D = true (latent) disease status, can be written as

$$P(T_1+T_2+) = Se_1Se_2p + (1 - Sp_1)(1 - Sp_2)(1 - p), \quad [2]$$

where p = prevalence of the latent disease. The probabilities of other combinations of test outcomes are expressed in the same way. Further details on the model specification can be found in Branscum et al. (2005).

We used OpenBUGS, version 3.0.8 (Thomas et al., 2006), for implementation of the latent class model and used uninformative Beta(1,1) priors, because no information was available on Se and Sp of cSCC and bacteriological culture in goats to serve as prior information. The Markov chain Monte Carlo chain was run for 12,000 iterations and the first 2,000 iterations were discarded as the burn-in phase. Three chains with different initial values were run simultaneously. Convergence was assessed by visual inspection of the time-series plots of the parameters. Autocorrelation was assessed by visual inspection of the plots and, if necessary, thinning was performed by using every tenth sample to improve mixing of the chains. The posterior distributions of the test properties and population prevalences were reported as the median and corresponding 95% posterior probability interval (PPI, which can be seen as the Bayesian analog of a confidence interval). Separate models were run for each of the 3 sampling occasions to be able to estimate test characteristics at different stages of lactation. To check if the estimates for Se and Sp were stable across the populations, we repeated the models from the first sampling moment 4 times and excluded the herds one by one.

For comparison, we also calculated the test characteristics of cSCC as compared with culture as the reference test. Estimates of Se and Sp and their corresponding 95% CI were calculated with the binconf function from the Hmisc library in R (version 2.11.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Descriptive Statistics

For 13 animals, the exact kidding date could not be retrieved after the animals were selected. Another

10 animals were deleted from the data set because they turned out to be more than 42 DIM at the first sampling occasion. Nine cases of clinical mastitis were recorded by the farmers and records of these animals were also excluded from all analyses. On the first, second, and third sampling occasion, respectively, 13, 19, and 28 animals were lost to follow up (culled, sold, or missing at time of sampling); 2, 3, and 4 animals, respectively, had missing DHI data and 6, 3, and 1 goats, respectively, had contaminated samples for one or both halves. Two goats had only 1 functional udder half throughout the study. Animals with missing DHI data, animals with only 1 functional half, and animals with contaminated samples were excluded from the animal-level analyses, but were included in the descriptive statistics at udder-half level, resulting in 384, 381, and 372 goats in the animal-level analyses and 394, 388, and 379 goats in the half-level statistics (for the first, second, and third sampling, respectively). Descriptive statistics of culture results and corresponding SCC values at udder-half level are shown in Table 2. Culture of major pathogens was rare, and the majority of major pathogens consisted of *Staph. aureus*. Minor pathogens (mostly *C. bovis* and CNS) were frequently isolated. *Corynebacterium bovis* isolates were almost exclusively present in 2 herds and had a very low prevalence in the other 2 herds, but CNS species had approximately the same prevalence in all 4 herds. *Mycoplasma bovis* was not detected in any of the samples.

Test Characteristics of cSCC

Latent class estimates of Se and Sp of cSCC on the first sampling occasion (4 to 42 DIM) are presented in Figure 1 as a function of the cut-off value for cSCC. Comparable graphs could be made for the other 2 sampling occasions, but with slightly higher Se and lower Sp in the late lactation sampling (170 to 285 DIM). As we expected, the *Staph. aureus* models yielded slightly lower Sp for cSCC than the major pathogen models did. Test characteristics for cSCC and bacteriological culture of *Staph. aureus* at a threshold level of $1,500 \times 10^3$ cells/mL and estimated prevalence in the 4 herds are displayed in Table 3, together with test characteristics of cSCC calculated with culture as reference test. Composite SCC was estimated to be less sensitive but more specific in the latent class models than in the models with culture as reference test. Both cSCC and bacteriological culture appeared to be highly specific tests, but Se of culture was estimated very low. Minor differences in Se and Sp were observed when the herds were excluded one by one from the models, but the general trends were very similar.

Table 2. Prevalence and SCC of udder halves infected with *Corynebacterium bovis*, CNS, a mixed infection of CNS and *C. bovis*, *Staphylococcus aureus*, esculin-positive cocci, gram-negative bacteria, *Bacillus* spp., *Streptococcus dysgalactiae*, or yeast, cultured from goats in 4 Dutch dairy herds, sampled on 3 occasions in lactation

DIM	Infection status	Udder halves (n)					SCC (10^3 cells/mL)		
		Herd					Geometric mean	First quartile	Third quartile
		All (%)	1	2	3	4			
4 to 42	Negative	448 (57)	123	60	97	168	156	49	426
	<i>C. bovis</i>	204 (26)	2	142	60	0	603	268	1,310
	CNS	99 (13)	48	5	30	16	709	348	1,648
	CNS + <i>C. bovis</i>	14 (2)	0	13	1	0	1,081	448	2,200
	<i>Staph. aureus</i> ¹	11 (1)	4	3	2	2	7,953	3,340	17,386
	Esculin-positive cocci ¹	1 (0)	0	0	1	0	9,362	9,362	9,362
	Gram-negative ¹	1 (0)	0	0	1	0	258	258	258
	<i>Strep. dysgalactiae</i> ¹	0 (0)	0	0	0	0			
	Yeast	0 (0)	0	0	0	0			
	<i>Bacillus</i> spp.	1 (0)	0	1	0	0	42	42	42
	Contaminated	7 (1)	2	5	0	0	310	80	413
	Total	786 (100)	179	229	192	186	296	75	861
44 to 84	Negative	455 (59)	113	83	94	165	107	38	262
	<i>C. bovis</i>	179 (23)	1	124	52	2	443	169	1,109
	CNS	112 (14)	53	8	32	19	717	334	1,757
	CNS + <i>C. bovis</i>	5 (1)	0	3	2	0	497	317	755
	<i>Staph. aureus</i> ¹	15 (2)	6	5	2	2	3,353	2,721	6,361
	Esculin-positive cocci ¹	1 (0)	0	0	1	0	1,334	1,334	1,334
	Gram-negative ¹	1 (0)	0	0	1	0	8,477	8,477	8,477
	<i>Strep. dysgalactiae</i> ¹	2 (0)	1	0	1	0	1,303	363	4,681
	Yeast	1 (0)	1	0	0	0	240	240	240
	<i>Bacillus</i> spp.	0 (0)	0	0	0	0			
	Contaminated	3 (0)	2	0	1	0	404	82	8,403
	Total	774 (100)	177	223	186	188	205	57	672
170 to 285	Negative	360 (48)	107	59	46	148	240	60	828
	<i>C. bovis</i>	257 (34)	3	144	107	3	575	224	1,362
	CNS	115 (15)	52	6	32	25	1,067	582	1,884
	CNS + <i>C. bovis</i>	4 (1)	0	3	1	0	997	906	1,126
	<i>Staph. aureus</i> ¹	11 (1)	4	3	2	2	4,534	3,924	8,150
	Esculin-positive cocci ¹	6 (1)	0	0	4	2	1,073	445	2,638
	Gram-negative ¹	1 (0)	0	0	1	0	1,239	1,239	1,239
	<i>Strep. dysgalactiae</i> ¹	1 (0)	1	0	0	0	2,826	2,826	2,826
	Yeast	0 (0)	0	0	0	0			
	<i>Bacillus</i> spp.	0 (0)	0	0	0	0			
	Contaminated	1 (0)	0	0	1	0	28	28	28
	Total	756 (100)	167	215	194	180	433	161	1,263

¹Major pathogen.

Test properties of cSCC for detecting both major and minor pathogens were found to be strongly dependent on parity, with increasing parity yielding higher Se and markedly lower Sp. To obtain comparable Se and Sp across parities, cut-off values of 250, 500, and $1,000 \times 10^3$ cells/mL for first, second, and third or higher parity, respectively, were needed. These thresholds however, yielded still moderate Se (about 0.70–0.80) and Sp (about 0.75–0.90). As in the other models, Se was higher and Sp was lower in late lactation than in early lactation.

DISCUSSION

Test Characteristics of cSCC

The objective of this paper was to estimate test characteristics of cSCC for detecting *Staph. aureus*-infected

goats. Using latent class analysis, we could estimate Se and Sp of both cSCC and bacteriological culture and the results indicated that culture has a low Se and almost perfect Sp, whereas cSCC has high Se and high Sp. At a threshold of $1,500 \times 10^3$ cells/mL, we observed higher Se and higher or comparable Sp than what has been reported in the literature at a threshold of $1,000 \times 10^3$ cells/mL (Table 1). Because *Staph. aureus* has many other reservoirs in goats than only the udder gland (Mørk et al., 2010), complete eradication of this pathogen from a herd may be difficult, but a good aim would be to obtain or maintain a low prevalence of *Staph. aureus* in the herd. Composite SCC may be a useful diagnostic screening tool to achieve this goal. Therefore, the test has to perform well in low-prevalence populations and must be cheap and easy to perform because of the usually large numbers of animals to be

Table 3. Estimate and 95% posterior probability interval (PPI) or CI of sensitivity (Se), specificity (Sp), and prevalence (P) of composite SCC at a threshold value of $1,500 \times 10^3$ cells/mL (cSCC1500) and bacteriological culture as tests for diagnosis of intramammary infection by *Staphylococcus aureus* at animal level, determined with latent class analysis and with bacteriological culture as reference test¹

Item	4 to 42 DIM	44 to 84 DIM	170 to 285 DIM
Latent class analysis, estimate (95% PPI)			
Se cSCC1500	0.86 (0.47 to 0.99)	0.81 (0.50 to 0.98)	0.91 (0.58 to 1.00)
Se culture	0.12 (0.05 to 0.25)	0.21 (0.10 to 0.43)	0.12 (0.05 to 0.29)
Sp cSCC1500	0.95 (0.87 to 1.00)	0.96 (0.9 to 1.00)	0.91 (0.79 to 0.99)
Sp culture	1.00 (0.98 to 1.00)	1.00 (0.98 to 1.00)	1.00 (0.98 to 1.00)
P _{herd1}	0.27 (0.14 to 0.52)	0.26 (0.13 to 0.45)	0.30 (0.13 to 0.52)
P _{herd2}	0.34 (0.19 to 0.62)	0.21 (0.08 to 0.37)	0.33 (0.14 to 0.56)
P _{herd3}	0.12 (0.02 to 0.28)	0.08 (0.02 to 0.18)	0.17 (0.04 to 0.33)
P _{herd4}	0.10 (0.02 to 0.24)	0.10 (0.02 to 0.22)	0.14 (0.03 to 0.30)
Culture as reference test, estimate (95% CI)			
Se cSCC1500	0.90 (0.60 to 0.99)	0.85 (0.58 to 0.96)	1.00 (0.72 to 1.00)
Sp cSCC1500	0.80 (0.76 to 0.84)	0.87 (0.83 to 0.90)	0.74 (0.70 to 0.79)

¹A total of 384 animals in 4 Dutch dairy goat herds were sampled 3 times during lactation at different DIM.

tested. Animals that tested positive in the screening can be subjected to a second test to confirm diagnosis. For screening purposes in a low prevalence population, a relatively sensitive test is needed, to ensure that only few infected animals remain unidentified, but perfect Se is not necessary for a successful control program. High specificity of the screening test is required, to ensure that not too many false-positives are identified in the screening. Essentially, a test with high positive predictive value (PPV) is needed in low-prevalence herds. Imagine a 1,000-goat herd, with a low *Staph. aureus* prevalence of 1%. A test with Se = 0.90 will detect 9 out of 10 infected animals. However, assuming Sp is also 0.90, these 9 truly infected animals will be diluted in 108 test-positive animals. This illustrates that a considerable amount of retesting is needed to find only a few truly infected animals. To use cSCC

as a first screening tool for detection of *Staph. aureus*-infected goats, given the prevalence in the herd is low, high Sp is more important than high Se. In our study, cSCC at a cut-off of $1,500 \times 10^3$ cells/mL had a Se of 0.86 and Sp of 0.95 in early lactation, corresponding to a negative predictive value (NPV) of 1.00 and a PPV of 0.26 at 2% prevalence. Therefore, cSCC can be used as a screening tool to detect *Staph. aureus*-infected goats. Figure 2 shows PPV and NPV for bacteriological culture and cSCC at 3 different cut-off values as a function of the prevalence. This figure illustrates that predictive values are strongly dependent on prevalence. The interpretation of the test results may, therefore, vary according to prior expectations on the prevalence in a herd. It may also be different in specific groups of animals that are expected to have a higher prevalence, such as higher-parity animals and animals in late lactation (Sánchez et al., 1999; Moroni et al., 2005). In this study we showed that the Se of culture was very low, which implies that serial combination with culture as confirmation test will still result in very low overall Se. This means that bacteriological culture cannot be used as confirmatory test. Another solution is, therefore, needed to identify false-positive results after screening with cSCC. One possible option could be to use a higher cut-off value for cSCC and sacrifice some Se in order to obtain higher Sp. Thus, we could eliminate the need to confirm findings, as bacteriological culture appears not to be suitable as a confirmatory test. Figure 2, however, shows that increasing the threshold from 1,500 to $2,500 \times 10^3$ cells/mL gives a relatively small increase in PPV but comes at the cost of a large decrease in NPV.

Figure 1. Plot of the sensitivity (Se) and specificity (Sp) of composite SCC (cSCC) for detecting *Staphylococcus aureus*-infected goats as a function of the cut-off value. The thin lines represent the upper and lower limits of the 95% posterior probability intervals.

Sensitivity of Bacteriological Culture

In our models, Se of culture for *Staph. aureus* was estimated to be very low (0.12 to 0.21). To our knowledge, no literature estimates of Se of culture are avail-

Figure 2. Plot of the positive predictive value (black lines) and negative predictive value (gray lines) as a function of the prevalence based on latent class analysis estimates of early lactation test characteristics of bacteriological culture [sensitivity (Se) = 0.12 and specificity (Sp) = 0.995], and composite SCC (cSCC) at threshold values of 250 (cSCC250; Se = 0.93, Sp = 0.71), 1,500 (cSCC1500; Se = 0.86, Sp = 0.95), and $2,500 \times 10^3$ cells/mL (cSCC2500; Se = 0.55, Sp = 0.98).

able for goats, but in cattle, Sanford et al. (2006) also found a relatively low Se for detecting major pathogens (0.62; 95% PPI = 0.41 to 0.94). In their study however, *Staph. aureus* comprised about one-third of the major pathogens, whereas the other major infections were caused by non-agalactiae streptococci and gram-negatives. The fact that we only looked at *Staph. aureus* may explain the even lower Se that we observed. From studies in cattle, we know that *Staph. aureus* is shedded intermittently (Sears et al., 1990) and, therefore, repeated samples over time give higher Se than a single sample (Buelow et al., 1996b). A larger inoculum also increases the Se of culture to detect *Staph. aureus*, as does preculture freezing and incubation (Buelow et al., 1996a; Sol et al., 2002; Artursson et al., 2010). In our study, no preculture freezing or incubation were practiced and we used an inoculum volume of 0.01 mL, corresponding to a Se of 0.19 (95% CI: 15.4 to 22.7) relative to the use of higher inoculum volumes and the use of preculture techniques, according to the study by Sol et al. (2002). Thus, due to the intermittent shedding pattern, the bacteria are not always present in a milk sample if the gland is infected, but even if the bacteria are present in the milk sample, the probability of detection through conventional culture is still very low. These observations in cattle may indicate that also in goats Se of culture for *Staph. aureus* would be low, as is suggested by our results. If that is true, the true prevalence of major pathogens in goat herds would be much higher than what is usually reported based on bacteriological culture. It would also explain the com-

monly reported findings of culture-negative results in goats with high SCC (Wilson et al., 1995; White and Hinckley, 1999; Min et al., 2007). Because of the limited number of culture-positive animals in our study, more research is needed to confirm our findings. Preferably, such studies should also include other tests in the latent class models, such as PCR for detection of the bacterial agents (Koskinen et al., 2009), or indirect mastitis tests such as electrical conductivity (Tangorra et al., 2010), N-acetyl- β -D-glucosaminidase (Maisi, 1990) or other enzymes (Katsoulos et al., 2010).

Disease Definition

An implicit assumption of the latent class models is that both tests that are used contain information about the same latent condition. In our case, we assume that this condition is *Staph. aureus* IMI, which can be defined as the presence of *Staph. aureus* in the udder gland. Bacteriological culture measures the presence of the bacterial agents directly, and is, therefore, in nature a good estimator of the disease of interest. Somatic cell count measures the immune response to the infection and is an indirect estimator of the disease of interest. Because SCC and culture are combined in the latent class model, the definition of the latent disease is based on both tests. This implicates that factors that affect SCC also contribute to the disease definition. The most important of these factors are parity and stage of lactation (Paape et al., 2001; Koop et al., 2009). Stratifying on these variables is therefore necessary, as we did in this study. Another factor that influences SCC in goats is caprine arthritis encephalitis virus (CAEV) infection (Sánchez et al., 2001; Turin et al., 2005). In our study, 3 out of the 4 herds were certified free from CAEV, and the fourth herd had no certificate, but clinical symptoms were not seen in the herd for years. Other factors affect SCC in goats to a limited extent, such as estrus (McDougall and Voermans, 2002; Moroni et al., 2007) and various stress factors like vaccination or acidosis (Lerondelle et al., 1992). The effect of these factors should, in theory, only affect the test properties of SCC, but because a latent class model is used, they also contribute to the definition of the latent disease. Therefore, these factors affect the prevalence of diseased animals and, thus, the estimated test properties of bacteriological culture. An important aspect here is the choice of cut-off values of SCC. Infections with *Staph. aureus* elicit a stronger SCC increase than do infections with minor pathogens. Nevertheless, minor pathogen infections may lead sometimes to SCC levels above the threshold for *Staph. aureus* and thus, contrib-

ute to the disease definition of *Staph. aureus* infection. This indicates that prevalence of *Staph. aureus* may be overestimated and, consequently, Se of culture may be underestimated in our models.

Model Assumptions

The first assumption of the latent class model is that the 2 tests are conditionally independent, given disease status. The 2 tests that we used in this study are based on 2 different biological mechanisms, namely the shedding of somatic cells (mainly neutrophils; Paae et al., 2001) and the presence of bacteria in milk, making conditional independence probable. Sanford et al. (2006) found no evidence for conditional covariance between cow-level California Mastitis Test, which is also based on somatic cells in milk and culture. Nevertheless, we can never be sure of complete absence of conditional dependence. After an example in Gardner et al. (2000), one can argue that early in the process of infection, the number of bacteria that is shed in the milk is low, and cell counts are not elevated yet, so false-negative results are more probable in early stages of infection for both tests. This is an indication of possible covariance of Se. It is more difficult to think of biological reasoning that might indicate the presence of covariance of Sp, but it cannot be completely ruled out. If conditional dependence is present in our study, we would expect it to be a positive correlation implying an overestimation of the test properties (Toft et al., 2005). This would mean that especially estimates of Se may have been overestimated. It is possible to model covariance in the latent class model, but that would give us 2 more parameters to estimate and we would need more than 2 tests to provide us with the degrees of freedom to keep the model identifiable or we would need to include prior information on some of the parameters, which was not available.

The second assumption of the latent class model is that Se and Sp of the tests is constant across all populations. To check this assumption, we repeated the models with exclusion of 1 of the 4 herds each time. Because this did not cause a large effect on the estimates, we can assume that this assumption was not violated. The largest effect was observed after excluding herd 2, increasing the early lactation estimate for Se of culture, as given in Table 3, from 0.12 to 0.20. The second largest effect was seen after excluding herd 1, lowering the estimate for Se of culture from 0.12 to 0.08 and the estimate for Se of cSCC (Table 3) from 0.86 to 0.74. All other estimates for Se and Sp changed less than 5% after exclusion of any of the herds.

The final assumption is that prevalence of disease should differ between populations. In our models, the differences between estimated prevalences were relatively small. This causes the wide 95% PPI for Se and prevalence (Toft et al., 2005). This becomes even worse because of the fact that the actual prevalence of major pathogens in this study was low, so the estimation of Se was based on a limited number of observations. Especially with higher cut-off values, the number of test-positive animals decreases, resulting in larger PPI, as can be seen in Figure 1. Specificity, on the other hand, could be estimated with high precision because of the large number of observations, but the problems to estimate Se correctly may also have affected the Sp estimates. Therefore, to obtain more precise results, future studies should try to obtain larger differences in higher-prevalence populations.

CONCLUSIONS

This study shows that cSCC can be used as a screening tool to detect *Staph. aureus*-infected animals, but that a confirmatory test is needed to identify false-positive results. Using latent class models, Se of bacteriological culture was estimated to be very low, making it unsuitable as a confirmatory test. Based on bacteriological culture alone, the apparent prevalence of *Staph. aureus*-infected goats was low, but with latent class models the prevalence of *Staph. aureus* was estimated to be much higher, indicating that *Staph. aureus* IMI may be an underestimated problem in dairy goat herds. More research is needed to confirm these findings.

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