Evaluation of the composite milk somatic cell count as a predictor of intramammary infection in dairy cattle

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ABSTRACT

The objectives of this study were (1) to evaluate the test characteristics and predictive values of quarter-composite milk somatic cell count (quarter-cSCC) values based on either a single observation or the geometric mean of multiple recordings as a predictor of intramammary infection (IMI) in lactating dairy cows; and (2) to explore to what extent herd prevalence of IMI and cow factors such as parity and stage of lactation affect them. A total of 780 single-quarter milk samples were collected from 195 dairy cows for bacteriologic culture at a single cross-sectional herd screening performed at 21 different dairy herds as part of different research projects. Additionally, monthly quarter-cSCC milk samples at test day were available as part of the Dairy Herd Improvement program. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated to differentiate cows infected with any pathogen and cows infected with major pathogens from uninfected cows. Different threshold values for quarter-cSCC, ranging between 50,000 and 500,000 cells/mL, were evaluated for all animals in the study, as well as for high- and low-prevalence herds, heifers and multiparous cows, and cows in early, mid, and late lactation. The overall Se and Sp at a threshold of 200,000 cells/mL for a single quarter-cSCC observation obtained closest to the time of bacteriologic culture were 44.3 and 87.3%, respectively, for cows infected with any pathogen, and 65.1 and 73.0%, respectively, for cows infected with major pathogens. The overall PPV and NPV at a threshold of 200,000 cells/mL for a single quarter-cSCC observation obtained closest to the time of bacteriologic culture were 89.9 and 38.1%, respectively, for cows infected with any pathogen, and 40.6% and 88.1%, respectively, for cows infected with

major pathogens. No major differences were observed between estimates of the test characteristics and predictive values of the quarter-cSCC criteria based on a single observation and the geometric mean of multiple observations. For IMI with any pathogen, the Se and PPV were higher in high-prevalence herds than in low-prevalence herds, particularly at thresholds of 50,000 and 100,000 cells/mL. For IMI with major pathogens, Sp was substantially higher in low-prevalence herds than in high-prevalence herds. Sensitivity was higher in multiparous cows than in heifers infected with any pathogen, more specifically at a threshold of 100,000 and 200,000 cells/mL. For cows in early and mid lactation infected with any pathogen, Sp was higher than for cows in late lactation using the single observation closest to the time of bacteriologic culture. The results suggest that the quarter-cSCC threshold value to select cows for bacteriologic culture to maximize the likelihood of finding the causative pathogen of IMI should depend on the group of pathogens one is interested in, the herd prevalence of subclinical mastitis, lactation stage, and the cow's parity.

Key words: dairy cow, infection status, predictive value, somatic cell count, test characteristics

INTRODUCTION

Worldwide, mastitis is still one of the most prevalent and costly diseases faced by the dairy industry (Dijkhuizen and Stelwagen, 1981; Trinidad et al., 1989; Laevens et al., 1997). Mastitis is usually the result of microorganisms, typically bacteria, penetrating the mammary gland via the teat canal and establishing IMI. The inflammatory response to IMI eventually results in either clinical or subclinical mastitis, of which the latter is the most common and costly form (Bradley et al., 2007; Huijps et al., 2010). The average cost of subclinical mastitis is estimated at €80 per cow per year (Huijps et al., 2010). The main financial losses are due to decreased milk production, and dairy producers supplying milk with a high bulk milk SCC may lose out on bonus payments or incur penalties.

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Quarter-composite milk SCC records (**quarter-cSCC**) are available on a regular basis for both producers and their advisors from Dairy Herd Improvement programs. Although quarter-cSCC is currently still considered the most suitable and accurate parameter for detecting the presence of IMI, separating infected from uninfected cows based on quarter-cSCC data is far from a perfect method (Dohoo and Meek, 1982; Viguir et al., 2009). Overall, the sensitivity (**Se**) and specificity (**Sp**) of quarter-cSCC as an indicator of IMI in at least 1 quarter, irrespective of the chosen threshold, range between 30 and 89%, and 60 and 90%, respectively (McDermott et al., 1982; Timms and Schultz, 1987; Reksen et al., 2008).

Both the Se and specificity for a specific SCC threshold are strongly driven by the cows' infection status and the virulence of the pathogen considered (i.e., major versus minor pathogens) (dos Reis et al., 2011). Besides IMI status, several other factors, such as parity, lactation stage, animal age and stress status, frequency of milking, and season have been associated with quarter-cSCC (Jaartsveld et al., 1983; Brolund, 1985; Harmon, 1994; Pyrl, 2003). Nevertheless, apart from a single study (Reksen et al., 2008) conducted at the quarter level, no information is available on how these factors affect the Se and specificity of quarter-cSCC as an indicator of IMI.

Early detection of cows with IMI, along with bacteriologic culture and implementation of specific measures based on the outcome, is still a cornerstone of mastitis control at the herd level (National Mastitis Council, 2001; Piepers et al., 2007). To motivate farmers and veterinarians to sample cows for bacteriologic culture, the probability of isolating a mastitis pathogen from the selected cows should be maximized: that is, the positive predictive value (**PPV**) should be as high as possible. The PPV for a specific quarter-cSCC threshold strongly depends on the prevalence of IMI in the tested population, and does not apply universally (Altman and Bland, 1994; National Mastitis Council, 2001; Dohoo et al., 2003). In Flanders (Belgium), a geometric mean of the last 3 monthly test days of SCC $\geq 250,000$ cells/mL is used as an indicator for cows to be cultured, because they are likely infected. Whether the proposed threshold is a good predictor of cows' IMI status and to what extent the PPV is also affected by other factors, such as parity or stage of lactation, has to our knowledge not yet been investigated.

Data from 3 longitudinal studies conducted in Flanders (Belgium) were combined into 1 data set (Piepers et al., 2010; Supr et al., 2011; Passchyn et al., 2013). The objectives of this study were (1) to determine the Se, Sp, PPV, and negative predictive value (**NPV**) of

several quarter-cSCC values based on either a single observation or the geometric mean of multiple recordings to differentiate between uninfected and infected cows in Flanders Belgium; and (2) to explore to what extent factors such as the herd prevalence of IMI, parity, and stage of lactation affect the Se, Sp, PPV, and NPV of quarter-cSCC at different thresholds. The best threshold was selected using the receiver operating characteristic (**ROC**) methodology as done by Detilleux et al. (1999); Se and Sp were assumed to be equally important in identifying cows with IMI causing subclinical mastitis.

MATERIALS AND METHODS

Data Origin and Herds

Data from 3 longitudinal studies conducted in Flanders (Belgium) were combined into 1 data set. Twelve herds from a first study were included in the current data set. Information on the herds used in this study has been described in detail elsewhere (Piepers et al., 2010). Briefly, the dairy herds were located in East Flanders within a radius of 30 km around Merelbeke, and the study was designed to gain more in-depth insights into the epidemiology of subclinical mastitis in fresh dairy heifers (Piepers et al., 2010, 2011). At the onset of the study, a cross-sectional screening was performed on each herd to determine the herd-level prevalence of IMI. In all herds, the cows calved year-round. The data set was further expanded with data from 6 dairy herds that participated in a second precisely conducted longitudinal study. All of these dairy herds were located in West Flanders, within a radius of 20 km around Torhout (Passchyn et al., 2013). Herd owners were approached for their willingness to participate in a clinical trial on the effect of systemic prepartum treatment of dairy heifers. Similar to the first study, quarter milk samples for bacteriologic culture were collected at the outset to determine the herd-level prevalence of IMI. In those herds as well, cows calved year-round. Another 3 herds were added that participated in a longitudinal study conducted to profile the distribution of CNS species causing IMI using molecular identification and to gain more insight in the pathogenic potential of CNS as a group and of the most prevalence species causing IMI (Supr et al., 2011). The dairy herds in this study were also located in East Flanders within a radius of 30 km around Merelbeke. In each dairy herd included in this study, a cohort of 25 animals was randomly selected within parity blocks (first, second, and third or more) at the outset, using the RAND function in Excel 2007 (Microsoft Corp., Redmond, WA). The data from the

Table 1. Overview of herd performance and cow characteristics

Variable	No.	%	Average	Minimum	Maximum
Herd performance					
Average herd size, no. of lactating cows	—	—	52	23	118
Average 305 d milk production, kg	—	—	8,652	4,036	10,369
Average herd SCC, cells/mL ¹	—	—	267,000	53,000	752,000
Cow characteristics					
Breed					
Black and White Holstein-Friesian	108	55.4	—	—	—
Red Holstein-Friesian	72	36.9	—	—	—
Red and White Holstein-Friesian cross-bred	15	7.7	—	—	—
Parity	—	—	3	1	9

¹Average of the monthly herd SCC as measured through the DHI program.

first sampling for bacteriologic culture performed on those herds were added to the data set used in the current study.

Herds that participated in 1 of the 3 studies needed to meet following requirements: (1) participation in the Dairy Herd Improvement program in Flanders (Flemish Cattle Breeding Association, Oosterzele, Belgium) on an annual basis, with an interval of 4 to 8 wk between 2 test days; (2) presence of a good animal identification and registration system; (3) record-keeping of all health issues on the farm (date of occurrence, identification of animal, diagnosis, and treatment). None of the herds was equipped with an automatic milking system.

More detailed information on herd performance and characteristics of the herds and cows included in the study can be found in Table 1.

Animals and Samples

A total of 780 single-quarter milk samples from 195 clinically healthy dairy cows were collected at a single cross-sectional herd screening at 21 commercial dairy herds performed between July 2006 and February 2009 as part of the research projects described above. The milk samples were collected aseptically by a veterinarian after disinfection of the teats and after the first streams of milk were discarded. Milk samples were immediately stored at 4°C, and then transported under cooled conditions to the laboratory (Milk Control Center, Lier, Belgium).

Additionally, 4- to 8-weekly quarter-composite milk samples at test day were available from each cow that was sampled for bacteriologic culture at the quarter level. The samples were collected by personnel from the local DHIA. All milk samples were stored with 0.02% Bronopol (Boots Company PLC, Nottingham, UK) at 4°C, and were transported to the laboratory (Milk Control Center). Only cows with at least 4 quarter-cSCC records before sampling for bacteriologic culture were eventually included in the study (n = 195). The

quarter-cSCC records were merged with the culture results.

Laboratory Analyses

Bacteriologic culture was done as previously described by the Milk Control Center (Piepers et al., 2010). Briefly, 0.01 mL of milk was plated on a blood-esculin agar (Oxoid, Erembodegem, Belgium; 1 plate per cow) and on a MacConkey's agar (1 plate per cow). All plates were incubated aerobically for 36 ± 12 h at 37 ± 1°C. A quarter was considered infected when the growth of ≥1 colony was detected, except for quarters infected with CNS and *Corynebacterium bovis*, which were considered infected when the growth of ≥2 colonies was detected. Samples yielding 3 or more different bacterial species were considered to be contaminated. For gram-positive cocci, catalase-tests were used to differentiate between catalase-positive staphylococci and catalase-negative streptococci. DNase testing, colony morphology, and hemolysis patterns were used to distinguish *Staphylococcus aureus* from CNS. Streptococci were subdivided into esculin-positive streptococci (*Streptococcus uberis*) and esculin-negative streptococci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*). *Corynebacterium bovis* has been distinguished from the catalase-positive staphylococci by colony morphology and Gram staining. *Trueperella pyogenes* has been distinguished from the catalase-negative streptococci based on growth characteristics (no growth visible after 24 h of incubation at 37°C), hemolysis patterns, and Gram staining. Coliforms, including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., were differentiated from each other and from other gram-negative bacteria based on the appearance on MacConkey's agar, KOH testing, triple sugar iron reactions, indole production, and motility.

Electronic counting was used to quantify SCC (Fossomatic 5000; Foss Electric, Hillerød, Denmark) in the Milk Control Center.

Table 2. Infection status of 195 dairy cows from 21 dairy herds in Flanders stratified by average herd SCC, parity, and stage of lactation

Variable	Infection status at cow level, no. (%)		
	Uninfected	Infected with minor pathogen(s)	Infected with major pathogen(s)
Average herd SCC			
Low-prevalence herd ¹	35 (46.7)	36 (48.0)	4 (5.3)
High-prevalence herd ²	20 (16.7)	61 (50.8)	39 (32.5)
Parity			
Heifer	19 (32.8)	31 (53.4)	8 (13.8)
Multiparous	36 (26.3)	66 (48.2)	35 (25.5)
Stage of lactation			
≤100 d	4 (26.7)	10 (66.7)	1 (6.7)
>100 and ≤199 d	21 (36.8)	24 (42.1)	12 (21.1)
≥200 d	30 (24.4)	63 (51.2)	30 (24.4)

¹Average herd SCC >200,000 cells/mL.²Average herd SCC ≤200,000 cells/mL.

Infection Status

Quarters. Quarters were allocated to 1 of 3 classes: uninfected, infected with a minor pathogen, and infected with a major pathogen. A quarter was considered uninfected if no bacteria were isolated. A quarter was considered infected with a minor pathogen if either CNS or *C. bovis* were isolated. A quarter was considered infected with major pathogens if *Staph. aureus*, *Strep. agalactiae*, esculin-positive streptococci, *Strep. dysgalactiae*, *T. pyogenes*, or gram-negative bacteria were isolated. If a quarter was infected with both major and minor pathogens, it was considered infected with major pathogens. Contaminated samples and quarters that could not be allocated to one of the above-mentioned classes were considered indefinable.

Cows. Quarter-level IMI information was aggregated at the cow level, classifying cows as uninfected, infected with a minor pathogen, or infected with a major pathogen. Cows with ≥1 indefinable quarter were excluded from the study. A cow was considered uninfected if all 4 quarters were uninfected. A cow was considered infected with a minor pathogens if ≥1 quarter was infected with a minor pathogen and none of the other quarters was infected with a major pathogen. A cow was considered infected with a major pathogen if ≥1 quarter was infected with a major pathogen.

Herd- and Cow-Level Variables

Several herd- and cow-level factors were available. Herd SCC was available from DHI data (i.e., average of all quarter-cSCC data at each test day). Herds with an average herd SCC >200,000 cells/mL between 2006 and 2009 were considered “high-prevalence herds” (HP herds), and herds with an average herd SCC ≤200,000 cells/mL were considered “low-prevalence herds” (LP

herds). Parity information was extracted from DHI records (heifers versus multiparous animals). Animals were classified as being in early lactation when ≤100 DIM, in mid lactation when >100 and ≤199 DIM, and in late lactation when ≥200 DIM at the time of bacteriologic culture. The percentage of cows infected with major pathogens was 32.5% in HP herds and 5.3% at LP herds. As well, 13.8% of heifers and 25.5% of multiparous cows were infected with major pathogens. Only 1 cow infected with major pathogens was <100 DIM. The percentage of cows infected with minor pathogens was equally distributed among HP and LP herds, among heifers and multiparous cows, and among the different stages of lactation (Table 2).

Test Characteristics and Predictive Values

The efficacy of several quarter-cSCC thresholds for differentiating infected from uninfected cows at the cow level was evaluated, calculating Se, Sp, PPV, NPV, and 95% CI, as described by Dohoo et al. (2003). The significance of differences in test characteristics and predictive values was assessed based on the overlap between 95% CI.

Two subsets of data were prepared from the main data set:

- (1) Assessment of the diagnostic properties of a single quarter-cSCC observation on average obtained at either 4 test days (**TD-4**; 135 d, range 86 to 216 d), 3 test days (**TD-3**, 98 d, range 55 to 180 d), 2 test days (**TD-2**, 56 d, range 20 to 140 d) or 1 test day (**TD-1**, 18 d, range 26 to –11 d) before bacteriologic culture.
- (2) Assessment of the diagnostic properties of the geometric mean quarter-cSCC obtained between

an average of 18 and 135 d before sampling for bacteriologic culture, using information from the 4 test days described above. The geometric mean of the last 2 quarter-cSCC records relative to the time of bacteriologic culture was further referred to as **GEO2**, the geometric mean of the last 3 quarter-cSCC records as **GEO3**, and the geometric mean of the last 4 quarter-cSCC records as **GEO4**, respectively.

For all subsets of data, ROC curves were constructed using Excel 2007 (Microsoft Corp.) to differentiate cows infected with major or minor pathogen(s) (i.e., any pathogen) from uninfected cows, as well as to differentiate cows infected with major pathogens only from cows that were uninfected or infected with minor pathogens for different quarter-cSCC thresholds ranging between 50,000 and 500,000 cells/mL. The ROC curve represented the fraction of true infected from the infected (Se) cows versus the fraction of false-positives from the uninfected ($1 - \text{Sp}$) cows using overall quarter-cSCC thresholds defined by Zweig and Campbell (1993).

For each single quarter-cSCC record and the geometric mean of multiple quarter-cSCC values, the quarter-cSCC threshold corresponding to the highest Se and Sp was identified by calculating the Youden's index from the different ROC curves. Youden's index was expressed

as the vertical distance between the ROC curve and the first bisector, and was defined as the difference between the true positive rate and the false-positive rate ($\text{Se} + \text{Sp} - 1$). The quarter-cSCC threshold with the highest Youden's index was considered to have the best ability to correctly discriminate between uninfected and infected cows (Youden, 1950; Greiner et al., 2000; Enrique et al., 2005).

Test characteristics and predictive values were calculated separately for HP and LP herds, for heifers and multiparous cows, and for cows in early, mid-, and late lactation cows for the quarter-cSCC criteria with the highest Youden's index for single observations and for the geometric mean of multiple recordings, separately.

RESULTS

Bacteriologic Culture

Quarter Level. Overall, 44% ($n = 343$) of the 779 quarters were infected (Table 3). The most frequently isolated mastitis pathogen was *C. bovis* (49.9% of infected samples), followed by *Staphylococcus* spp. (24.5%), *Staph. aureus* (12.8%), and esculin-positive streptococci (7.6%).

Cow Level. Approximately 72% of the cows ($n = 140$) were infected with either a major or minor patho-

Table 3. Prevalence and distribution of mastitis pathogens isolated from 195 dairy cows from 21 dairy herds in Flanders

Pathogen	Level ¹	No.	% of total	% of infected	Quarter-composite milk SCC ²		
					Average	Minimum	Maximum
Uninfected	C	55	28.2	—	56	7	1,408
	Q	436	56.0	—	—	—	—
Infected with:							
<i>Staphylococcus aureus</i>	C	20	10.3	14.3	409	30	2,567
	Q	44	5.6	12.8	—	—	—
Esculin-positive streptococci	C	14	7.2	10.0	149	26	621
	Q	26	3.3	7.6	—	—	—
Esculin-negative streptococci	C	2	1.0	1.4	418	236	739
	Q	3	0.4	0.8	—	—	—
Mixed major pathogens ³	C	7	3.6	5.0	408	43	2,520
	Q	4	0.5	1.2	—	—	—
<i>Corynebacterium bovis</i>	C	43	22.1	30.7	140	58	236
	Q	171	22.0	49.8	—	—	—
<i>Staphylococcus</i> spp.	C	36	18.5	25.7	111	22	7,739
	Q	84	10.8	24.5	—	—	—
Mixed minor pathogens ⁴	C	18	9.2	12.9	160	22	1,039
	Q	8	1.0	2.3	—	—	—
Other ⁵	C	3	1.5	2.1	350	42	2,514
	Q	3	0.4	0.9	—	—	—

¹C= cow; Q = quarter.

²Quarter-composite milk SCC $\times$ 1,000 cells/mL.

³Mixed major pathogen per quarter: Esculin-positive streptococci/gram-negative bacteria ($n = 2$); esculin-positive streptococci/*Staphylococcus* spp. ($n = 1$); *Corynebacterium bovis*/esculin-positive streptococci ($n = 1$).

⁴Mixed minor pathogens per quarter: *Corynebacterium bovis*/*Bacillus* spp. ($n = 1$); *Staphylococcus* spp./ *Corynebacterium bovis* ($n = 7$).

⁵Other pathogens: *Trueperella pyogenes* ($n = 1$); gram-negative bacteria ($n = 2$).

gen in at least 1 quarter (Table 3). Almost 50% ($n = 97$) of cows were infected with either *Staphylococcus* spp. (44%; $n = 36$) or *C. bovis* (37%; $n = 43$). Esculin-positive streptococci accounted for 33% ($n = 14$) of cows infected with major pathogens, and *Staph. aureus* accounted for 47% ($n = 20$) (Table 3).

Diagnostic Characteristics

Se and Sp. The Se decreased and Sp increased for all single TD-1, 2, 3, and 4, and GEO-2, 3, and 4 criteria when a higher threshold level of quarter-cSCC was used to detect cows infected with any pathogen or major pathogens. For a single observation, the highest Sp for IMI with any pathogen and IMI with major pathogens was observed using TD-1 at thresholds of 50,000 and 200,000 cells/mL, respectively. The Se for IMI with major pathogens using TD-2 was equal to that obtained using TD-1. For the geometric mean of multiple observations, the highest Se was obtained using GEO2 at a threshold of 50,000 cells/mL for both IMI with any pathogen and IMI with major pathogens. For a single observation, the highest Sp for IMI with any pathogen was obtained using TD-2 and for IMI with major pathogens using TD-3, both at a threshold of 500,000 cells/mL. For multiple observations, the highest Sp was obtained using GEO3 for IMI with any pathogen and GEO-4 for IMI with major pathogens, both at a threshold of 500,000 cells/mL. Still, for IMI with any pathogen, the Sp obtained using GEO-4 was equal to the Sp obtained using GEO-3. Overall, based on the overall 95% CI, differences among the various quarter-cSCC criteria at a certain threshold were not significant.

PPV and NPV Based on overall 95% CI, PPV increased and NPV decreased for TD-1, 2, 3, and 4, and for GEO-2, 3, and 4 when a higher threshold level of quarter-cSCC was used to detect cows infected with any pathogen or major pathogens. For a single observation, the highest PPV was obtained using TD-2 for IMI with any pathogen and using TD-3 for IMI with major pathogens, both at a threshold of 500,000 cells/mL. For multiple observations, the highest PPV for IMI with any pathogen and IMI with major pathogens was obtained using GEO3 at a threshold of 500,000 cells/mL. For a single observation, the highest NPV for IMI with any pathogen was obtained using TD-1 and TD-2 for IMI with major pathogens, both at a threshold of 50,000 cells/mL. For multiple observations, the highest NPV for IMI with any pathogen and IMI with major pathogens was obtained using GEO2 at a threshold of 50,000 cells/mL. Based on the overlap of 95% CI, the differences among the various quarter-cSCC criteria at a certain threshold were not significant.

Youden's Index. For a single observation, the highest Youden's index was obtained using TD-1 for both IMI with any pathogen and IMI with major pathogens, at thresholds of 50,000 cells/mL and 200,000 cells/mL, respectively (Tables 4 and 5). For multiple observations, the highest Youden's index was obtained using GEO4 for IMI with any pathogen at a threshold of 100,000 cells/mL and GEO2 for IMI with major pathogens at a threshold of 200,000 cells/mL. However, the differences in Youden's index between the different quarter-cSCC criteria were small (Tables 6 and 7).

Herd- and Cow-Level Variables. For IMI with any pathogen, Se was higher in HP herds than in LP herds at all tested thresholds (i.e., 50,000, 100,000, or 200,000 cells/mL) for TD-1 and GEO4 (Table 8). For IMI with major pathogens, Sp was substantially higher in LP herds than in HP herds for TD-1 and GEO2 (Table 9). For TD-1, Se was higher in multiparous cows than in heifers infected with any pathogen, more specifically at thresholds of 100,000 and 200,000 cells/mL (Table 8). Specificity for cows infected with any pathogen was higher for heifers than for multiparous cows for GEO4, particularly at a threshold of 50,000 cells/mL (Table 10). For cows in early and mid lactation and infected with any pathogen, Sp was higher than for cows in late lactation for TD-1 (Table 8). We observed a similar trend for GEO4, although it was less pronounced (Table 10). For IMI with any pathogen, the PPV was higher for HP herds than for LP herds for TD-1 and GEO4, particularly at thresholds of 50,000 and 100,000 cells/mL (Tables 8 and 10). A similar trend was observed for IMI with major pathogens for both TD-1 and GEO2 (Tables 9 and 11). The PPV for multiparous cows infected with major pathogens was higher than for heifers infected with major pathogens for both TD-1 and GEO2 (Tables 9 and 11), particularly at thresholds of 50,000 cells/mL.

DISCUSSION

This study aimed at determining optimal SCC threshold values for detecting IMI in a sampled population of Flemish dairy cows. Quarter IMI status was defined based on single-quarter milk samples, mimicking the sampling strategy used by farmers and veterinarians in the field. The data used in this study were somewhat unique in they came from 21 commercial dairy herds. The percentage of infected cows and quarters in our study was higher than the 41.1 and 17.0% obtained by Piepers et al. (2007) using a large data set collected between 2000 and 2002. The latter difference in prevalence between both studies might imply that our results overestimate the true PPV and underestimate the true NPV in Flemish dairy herds. Most other studies seek-

Table 4. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and Youden's index (Y) of diagnostic tests with a single composite milk SCC (quarter-cSCC) as a predictor of cows infected with any pathogen when the threshold was set at different quarter-cSCC values

Quarter-cSCC criteria/threshold ¹	Cow infected with any pathogen											
	Se			Sp			PPV			NPV		
	%	95% CI		%	95% CI		%	95% CI		%	95% CI	Y, %
TD-4												
≥50	64.3	56.3–72.2	65.5	52.9–78.0	82.6	75.4–89.7	41.8	31.4–52.3				29.7
≥100	48.6	40.3–56.9	83.6	73.9–93.4	88.3	81.1–95.5	38.9	30.2–47.8				32.2
≥200	32.1	24.4–39.9	89.1	80.9–97.3	88.2	79.4–97.1	34.0	26.3–41.8				21.2
≥250	27.1	19.8–34.5	90.9	83.3–98.5	88.4	78.8–98.0	32.8	25.4–40.4				18.1
≥500	14.3	8.5–20.1	94.5	88.5–100	87.0	73.2–100	30.2	23.4–37.1				8.8
TD-3												
≥50	75.7	68.6–82.8	58.2	45.1–71.2	82.2	75.6–88.8	48.4	36.4–60.5				33.9
≥100	54.3	46.0–62.5	74.5	63.0–86.1	84.4	77.7–91.9	39.0	29.7–48.4				28.8
≥200	36.4	28.5–44.4	92.7	85.9–99.6	92.7	85.9–99.6	36.4	28.5–44.4				29.2
≥250	30.0	22.4–37.6	92.7	85.9–99.6	91.3	83.2–99.4	34.2	26.6–41.8				22.7
≥500	15.7	9.7–21.7	96.4	91.4–100	91.7	80.6–100	30.9	24.1–37.9				12.1
TD-2												
≥50	77.1	70.2–84.1	54.5	41.4–67.7	81.2	74.6–87.8	48.3	35.9–60.8				31.7
≥100	60.7	52.6–68.8	76.4	65.1–87.6	86.7	80.0–93.5	43.3	35.9–60.8				37.1
≥200	35.7	27.8–43.7	92.7	85.9–99.6	92.6	85.6–99.6	36.1	28.2–44.1				28.4
≥250	29.3	21.7–36.8	94.5	88.5–100.5	93.2	85.7–100	34.4	26.9–42.0				23.8
≥500	15.0	9.1–20.9	98.2	94.7–100	95.5	86.8–100	31.2	24.3–38.1				13.2
TD-1												
≥50	84.3	78.3–90.3	56.4	43.3–69.5	83.1	76.9–89.3	58.4	45.2–71.8				40.6
≥100	67.1	59.4–74.9	69.1	56.9–81.3	84.7	78.0–91.4	45.2	34.6–55.9				36.2
≥200	44.3	36.1–52.5	87.3	78.5–96.1	89.9	82.7–97.0	38.1	29.6–46.6				31.6
≥250	36.4	28.5–44.4	94.5	88.5–100	94.4	88.3–100	36.8	28.9–44.8				31.0
≥500	20.0	13.4–26.6	96.4	91.4–100	93.3	84.4–100	32.1	25.0–39.2				16.4

¹TD-4, TD-3, TD-2 = tests conducted for the fourth, third, and second quarter-cSCC record, respectively, relative to the time of bacteriologic culture; TD-1 = test conducted for the quarter-cSCC record closest to the time of bacteriologic culture. Thresholds are the quarter-cSCC ($\times 1,000$ cells/mL).

Table 5. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and Youden's index (Y) of diagnostic tests with a single composite milk SCC (quarter-cSCC) as predictor of cows with a major pathogen(s) when the threshold was set at different SCC values

Quarter-cSCC criteria/threshold ¹	Cows infected with a major pathogen(s)									
	Se		Sp		PPV		NPV		Y, %	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	Y, %
TD-4	72.1	58.7–85.5	48.7	40.7–56.6	28.4	20.0–36.9	86.0	78.7–93.4	86.0	20.8
	58.1	43.4–72.9	65.8	58.2–73.3	32.5	22.0–42.9	84.7	78.3–91.2	84.7	23.9
	48.8	33.9–63.8	80.3	73.9–86.6	41.2	27.7–54.7	84.7	78.8–90.6	84.7	29.1
	44.2	29.3–59.0	84.2	78.4–90.0	44.4	29.3–59.0	84.2	78.4–90.0	84.2	28.4
	23.3	10.6–35.9	91.4	87.0–95.9	43.5	23.2–63.7	80.8	74.9–86.7	80.8	14.7
TD-3	76.7	64.1–89.4	36.8	29.2–44.5	25.6	18.1–33.1	84.8	76.2–93.5	84.8	13.6
	60.5	45.9–75.1	57.9	50.0–65.7	28.9	19.5–38.3	83.8	76.8–90.9	83.8	18.4
	46.5	31.6–61.4	77.0	70.3–83.7	36.4	23.7–49.1	83.5	77.4–89.7	83.5	23.5
	39.5	24.9–54.1	80.9	74.7–87.2	37.0	23.0–50.9	82.5	76.5–88.6	82.5	20.5
	27.9	14.5–41.3	92.1	87.8–96.4	50.0	30.0–70.0	81.8	76.1–87.6	81.8	20.0
TD-2	90.7	82.0–99.4	38.2	30.4–45.9	29.3	21.6–37.1	93.5	87.4–99.7	93.5	28.9
	69.8	56.0–83.5	55.3	47.4–63.2	30.6	21.5–39.7	86.6	79.8–93.4	86.6	25.0
	51.2	36.2–66.1	78.9	72.5–85.4	40.7	27.6–53.8	85.1	79.2–91.0	85.1	30.1
	39.5	24.9–54.1	82.2	76.2–88.3	38.6	24.2–53.0	82.7	76.8–88.0	82.7	21.8
	16.3	05.2–27.3	90.1	85.4–94.9	31.8	12.4–51.3	79.1	73.1–85.2	79.1	6.4
TD-1	90.7	82.0–99.4	32.2	24.8–39.7	27.5	20.1–34.8	92.4	85.3–99.6	92.4	22.9
	86.0	75.7–96.4	51.3	43.4–59.3	33.3	24.6–42.1	92.8	87.3–98.4	92.8	37.4
	65.1	50.9–79.4	73.0	66.0–80.1	40.6	29.0–52.2	88.1	82.4–93.7	88.1	38.1
	55.8	41.0–70.7	80.3	73.9–86.6	44.4	31.2–57.7	86.5	80.9–92.2	86.5	36.1
	34.9	20.6–49.1	90.1	85.4–94.9	50.0	32.1–67.9	83.0	77.7–88.8	83.0	25.0

¹TD-4, TD-3, TD-2 = tests conducted for the fourth, third, and second quarter-cSCC record, respectively, relative to the time of bacteriologic culture; TD-1 = test conducted for the quarter-cSCC record closest to the time of bacteriologic culture. Thresholds are the quarter-cSCC ($\times 1,000$ cells/mL).

Table 6. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and Youden's index (Y) of diagnostic tests with the geometric mean composite milk SCC (quarter-cSCC) as predictor of cows infected with any pathogen when the threshold was set at different quarter-cSCC values

Quarter-cSCC criteria/threshold ¹	Cows infected with any pathogen									
	Se		Sp		PPV		NPV		Y, %	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI		
GEO2										
≥50	83.6	77.4–89.7	54.5	41.4–67.7	82.4	76.1–88.7	56.6	43.3–69.9	38.1	
≥100	64.3	56.3–72.2	69.1	56.9–81.3	84.1	77.7–91.0	43.1	32.8–53.5	33.4	
≥200	43.6	35.4–51.8	94.5	88.5–100	95.3	90.1–100	39.6	31.3–48.1	38.1	
≥250	31.4	23.7–39.1	94.5	88.5–100	93.6	86.6–100	35.1	27.4–42.8	26.0	
≥500	15.0	09.1–20.9	96.4	91.4–100	91.3	79.8–100	30.8	23.9–37.7	11.4	
GEO3										
≥50	80.7	74.2–87.2	54.5	41.1–67.7	81.9	75.5–88.3	52.6	39.7–65.6	35.3	
≥100	62.1	54.1–70.2	74.5	63.0–86.1	86.1	79.4–92.9	43.6	33.6–53.6	36.7	
≥200	37.1	29.1–45.1	92.7	85.9–99.6	92.9	86.1–99.6	36.6	28.7–44.7	29.9	
≥250	29.3	21.7–36.8	92.7	85.9–99.6	91.1	82.8–99.4	34.0	26.4–41.6	22.0	
≥500	15.7	9.7–21.7	98.2	94.7–100	95.7	87.3–100	31.4	24.5–38.3	13.9	
GEO4										
≥50	80.0	73.4–86.6	54.5	41.4–67.7	81.8	75.3–88.2	51.7	38.9–64.6	34.5	
≥100	61.4	53.4–69.5	78.2	67.3–89.1	87.8	81.3–94.2	44.3	34.4–54.2	39.6	
≥200	36.4	28.5–44.4	94.5	88.5–100	94.4	88.3–100	36.8	28.9–44.8	31.0	
≥250	27.9	20.4–35.3	94.5	88.5–100	92.9	85.1–100	33.9	26.5–41.5	22.4	
≥500	12.9	7.3–18.4	98.2	94.7–100	94.7	84.7–100	30.6	23.9–37.5	11.0	

¹GEO2, GEO3, and GEO4 = tests conducted for the geometric mean of the last 2, 3, and 4 quarter-cSCC records, respectively, relative to the time of bacteriologic culture. Thresholds are the quarter-cSCC ($\times 1,000$ cells/mL).

Table 7. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and Youden's index (Y) of diagnostic tests with the geometric mean quarter-composite milk SCC (quarter-cSCC) as predictor of cows infected with major pathogens when the threshold was set at different quarter-cSCC values

Quarter-cSCC criteria/threshold ¹	Cows infected with major pathogens							
	Se		Sp		PPV		NPV	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
GEO2								
>50	95.3	89.1–100	33.6	26.0–41.1	28.9	21.4–36.3	96.2	91.1–100
>100	86.0	75.7–96.4	53.9	46.0–61.9	34.6	25.6–43.6	93.1	87.9–98.4
>200	65.1	50.9–79.4	76.3	69.6–83.1	43.8	31.6–55.9	88.5	83.1–94.0
>250	48.8	33.9–63.8	82.9	76.9–88.9	44.7	30.5–58.9	85.1	79.4–90.0
>500	20.9	8.8–33.1	90.8	86.2–95.4	39.1	19.2–59.1	80.2	74.3–86.2
GEO3								
>50	93.0	85.4–100	35.5	27.9–43.1	29.0	21.4–36.6	94.7	88.9–100
>100	79.1	66.9–91.2	55.9	48.0–63.8	33.7	24.4–42.9	90.4	84.5–96.4
>200	48.8	33.9–63.8	77.0	70.3–83.7	37.5	24.8–50.2	84.1	78.1–90.2
>250	39.5	24.9–54.1	81.6	75.4–87.7	37.8	23.6–51.9	82.6	76.6–88.7
>500	23.3	10.6–35.9	91.4	87.0–95.9	43.5	23.2–63.7	80.8	74.9–86.7
GEO4								
>50	93.0	85.4–100	36.2	28.5–43.8	29.2	21.6–36.8	94.8	89.1–100
>100	74.4	61.4–87.5	56.6	48.7–64.5	32.7	23.4–41.9	88.6	82.3–95.0
>200	51.2	36.2–66.1	78.9	72.5–85.4	40.7	27.6–53.8	85.1	79.2–91.0
>250	41.9	27.1–56.6	84.2	78.4–90.0	42.9	27.9–57.8	83.6	77.8–89.5
>500	18.6	7.0–30.2	92.8	88.6–96.9	42.1	19.9–64.3	80.1	74.2–86.0

¹GEO2, GEO3, and GEO4 = tests conducted for the geometric mean of the last 2, 3, and 4 quarter-cSCC records, respectively, relative to the time of bacteriologic culture. Thresholds are the quarter-cSCC ($\times 1,000$ cells/mL).

Table 8. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests with the quarter-composite milk SCC (TD-1¹) as predictor of cows infected with any pathogen when threshold values were set at $\geq 50,000$, $\geq 100,000$, and $\geq 200,000$ cells/mL stratified by the average herd SCC, parity, and stage of lactation

Stratum	No.	Quarter-cSCC	Cows infected with any pathogen					
			Se			Sp		
			%	95% CI	%	95% CI	%	95% CI
All cows	195							
		$\geq 50,000$	84.3	78.3–90.3	56.4	43.3–69.5	83.1	76.9–89.3
		$\geq 100,000$	67.1	59.3–74.9	69.0	56.8–81.3	84.6	77.9–91.3
		$\geq 200,000$	44.3	36.1–52.5	87.3	78.5–96.1	89.9	82.7–97.0
Average herd SCC	120							
High-prevalence herd ²		$\geq 50,000$	89.0	82.9–95.1	55.0	33.2–76.8	90.8	85.1–96.5
		$\geq 100,000$	79.0	71.0–87.0	70.0	49.9–90.1	92.9	87.5–100
		$\geq 200,000$	54.0	44.2–63.8	85.0	69.4–100	94.7	88.9–100
Low-prevalence herd ³	75							
		$\geq 50,000$	72.5	58.7–86.3	57.1	40.7–73.5	65.9	51.9–79.9
		$\geq 100,000$	37.5	22.5–52.5	68.6	53.2–84.0	57.7	38.7–71.5
		$\geq 200,000$	20.0	7.6–32.4	88.6	78.0–99.1	66.7	40.0–93.3
Parity	58							
Heifers		$\geq 50,000$	76.9	63.7–90.1	68.4	47.5–89.3	83.3	71.2–95.5
		$\geq 100,000$	48.7	33.3–64.4	78.9	60.6–97.3	82.6	67.1–95.2
		$\geq 200,000$	25.6	11.9–39.3	84.2	67.8–100	76.9	54.0–99.8
Multiparous	137							
		$\geq 50,000$	87.1	80.6–93.7	50.0	33.7–66.3	83.0	75.9–90.2
		$\geq 100,000$	74.3	65.7–82.8	63.9	48.2–79.6	85.5	77.8–95.2
		$\geq 200,000$	51.5	41.7–61.2	88.9	78.6–99.2	92.9	86.1–99.6
DIM	15							
≤ 100		$\geq 50,000$	63.6	35.2–92.1	75.0	32.6–100	87.5	64.6–100
		$\geq 100,000$	36.4	7.9–64.8	100	100–100	100	100–100
		$\geq 200,000$	36.4	7.9–64.8	100	100–100	100	100–100
> 100 and ≤ 199	57							
		$\geq 50,000$	66.7	51.3–82.1	71.4	52.1–90.8	80.0	65.7–94.3
		$\geq 100,000$	52.8	36.5–69.1	81.0	64.2–97.7	82.6	67.1–95.3
		$\geq 200,000$	33.3	17.9–48.7	95.2	86.1–100	92.3	77.8–100
≥ 200	123							
		$\geq 50,000$	93.5	88.6–98.5	43.3	25.6–61.1	83.7	76.5–90.8
		$\geq 100,000$	76.3	67.7–85.0	56.7	38.9–74.4	84.5	76.8–95.9
		$\geq 200,000$	49.5	39.3–59.6	80.0	65.7–94.3	88.5	79.8–97.1

¹Test was conducted for the quarter-cSCC record closest to the time of bacteriologic culture.

²Average herd SCC $> 200,000$ cells/mL.

³Average herd SCC $\leq 200,000$ cells/mL.

Table 9. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests with the quarter-composite milk SCC (TD-1¹) as predictor of cows infected with major pathogens when threshold values were set at $\geq 50,000$, $\geq 100,000$, and $\geq 200,000$ cells/mL stratified by the average herd SCC, parity, and stage of lactation

Stratum	No.	Quarter-cSCC	Cow infected with major pathogen					
			Se		Sp		PPV	
			%	95% CI	%	95% CI	%	95% CI
All cows	195	$\geq 50,000$	90.7	82.0–99.4	32.2	24.8–39.7	27.5	20.1–34.8
		$\geq 100,000$	86.0	75.7–96.4	51.3	43.4–59.3	33.3	24.6–43.4
		$\geq 200,000$	65.1	50.9–79.4	73.0	66.0–80.1	40.6	29.0–52.2
Average herd SCC	120	$\geq 50,000$	92.3	83.9–100	23.5	14.2–32.7	36.7	27.2–46.3
High-prevalence herd ²		$\geq 100,000$	89.7	80.2–99.3	38.3	27.7–48.9	41.2	30.7–57.5
		$\geq 200,000$	66.7	51.9–81.5	61.7	51.1–72.3	45.6	32.7–58.5
Low-prevalence herd ³	75	$\geq 50,000$	75.0	32.6–100	42.3	30.8–53.7	6.8	0.00–14.3
		$\geq 100,000$	50.0	1.0–99.0	66.2	55.2–77.2	7.7	0.00–15.1
		$\geq 200,000$	50.0	1.0–99.0	85.9	77.8–94.0	16.7	0.00–37.8
Parity	58	$\geq 50,000$	62.5	29.0–96.0	38.0	24.5–51.5	13.9	2.60–25.2
Heifers		$\geq 100,000$	62.5	29.0–96.0	64.0	50.7–77.3	21.7	4.9–35.4
		$\geq 200,000$	37.5	4.0–71.0	80.0	68.9–91.1	23.1	0.20–46.0
Multiparous	137	$\geq 50,000$	97.1	91.6–100	29.4	20.6–38.3	32.1	23.2–41.0
		$\geq 100,000$	91.4	82.2–100	45.1	35.4–54.8	36.4	26.3–49.8
		$\geq 200,000$	71.4	56.5–86.4	69.6	60.7–78.5	44.6	31.6–57.7
DIM	15	$\geq 50,000$	100	100–100	50.0	23.8–76.2	12.5	0.00–35.4
≤ 100		$\geq 100,000$	100	100–100	78.5	57.0–100	25.0	0.00–67.4
		$\geq 200,000$	100	100–100	78.6	57.1–100	25.0	0.00–67.4
> 100 and ≤ 199	57	$\geq 50,000$	75.0	50.5–99.5	53.3	38.8–67.9	30.0	13.6–46.4
		$\geq 100,000$	66.6	39.9–93.3	66.6	52.8–80.4	34.7	15.3–54.2
		$\geq 200,000$	58.3	30.4–86.2	86.7	76.7–96.6	53.8	26.7–80.9
≥ 200	123	$\geq 50,000$	96.7	90.2–100	19.4	11.3–27.4	27.9	19.3–36.5
		$\geq 100,000$	93.3	84.4–100	39.7	29.8–49.7	33.3	23.2–43.4
		$\geq 200,000$	66.7	49.8–83.5	65.6	55.9–75.2	38.5	25.2–51.7

¹Test was conducted for the quarter-cSCC record closest to the time of bacteriologic culture.

²Average herd SCC $> 200,000$ cells/mL.

³Average herd SCC $\leq 200,000$ cells/mL.

Table 10. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests with the geometric mean quarter-composite milk SCC (GEO4) as predictor of the cow's infection status when threshold values were set at $\geq 50,000$, $\geq 100,000$, and $\geq 200,000$ cells/mL stratified by average herd SCC, parity, and stage of lactation

Stratum	No.	Quarter-cSCC	Cows infected by any pathogen								
			Se		Sp		PPV		NPV		
			%	95% CI	%	95% CI	%	95% CI	%	95% CI	
All cows	195										
		≥50,000	80.0	73.3–86.6	54.5	41.3–67.7	81.7	75.2–88.2	51.7	38.8–64.5	
		≥100,000	61.4	53.3–69.4	78.1	67.2–89.1	87.7	81.2–94.2	44.3	34.4–54.2	
		≥200,000	36.4	28.4–44.4	94.5	88.5–100	94.4	88.3–100	36.8	28.9–44.8	
Average herd SCC	120										
High-prevalence herd ²		≥50,000	90.0	84.1–95.8	50.0	28.0–71.9	90.0	84.1–95.8	50.0	28.0–71.9	
		≥100,000	72.0	63.2–80.8	75.0	56.0–93.9	93.5	88.0–99.0	34.8	20.6–49.1	
		≥200,000	45.0	35.2–54.7	90.0	76.8–100	95.7	89.9–100	24.6	14.7–34.5	
Low-prevalence herd ³	75										
		≥50,000	55.0	39.5–70.4	57.1	40.7–73.5	59.4	43.6–75.2	52.6	36.7–68.5	
		≥100,000	35.0	20.2–49.7	80.0	66.7–93.2	66.6	46.5–86.8	51.8	38.5–65.1	
		≥200,000	15.0	3.93–26.0	97.1	91.6–100	85.7	59.7–100	50.0	38.1–61.8	
Parity	58										
Heifers		≥50,000	71.7	57.6–85.9	73.6	53.8–93.4	84.8	72.6–97.0	56.0	36.5–75.4	
		≥100,000	51.2	35.5–66.9	78.9	60.6–97.2	83.3	68.4–98.2	44.1	27.4–60.8	
		≥200,000	28.2	14.0–42.3	89.4	75.6–100	84.6	65.0–100	37.7	23.6–51.9	
Multiparous	137										
		≥50,000	83.1	75.8–90.4	44.4	28.2–60.6	80.7	73.1–88.3	48.4	31.4–65.5	
		≥100,000	65.3	56.0–74.6	77.7	64.2–91.3	89.1	82.1–96.2	44.4	32.1–56.7	
		≥200,000	39.6	30.0–49.1	97.2	91.8–100	97.5	92.8–100	36.4	26.8–46.0	
DIM	15										
≤100		≥50,000	81.8	59.0–100	75.0	32.5–100	90.0	71.4–100	60.0	17.0–100	
		≥100,000	72.7	46.4–99.0	75.0	32.5–100	88.8	68.3–100	50.0	9.99–90.0	
		≥200,000	27.2	0.95–53.5	100	100–100	100	100–100	33.3	6.66–60.0	
>100 and ≤199	57										
		≥50,000	69.4	54.4–84.4	71.4	52.1–90.7	80.6	66.7–94.5	57.6	38.7–76.6	
		≥100,000	44.4	28.2–60.6	90.4	77.9–100	88.8	74.3–100	48.7	33.0–64.4	
		≥200,000	25.0	10.8–39.1	100	100–100	100	100–100	43.7	29.7–57.7	
≥200	123										
		≥50,000	83.8	76.4–91.3	40.0	22.4–57.5	81.2	73.4–89.0	44.4	25.7–63.1	
		≥100,000	66.6	57.0–76.2	70.0	53.6–86.4	87.3	79.5–95.0	40.3	27.0–53.7	
		≥200,000	41.9	31.9–51.9	90.0	79.2–100	92.8	85.0–100	33.3	23.0–43.6	

¹Test was conducted for the geometric mean of the last 4 quarter-cSCC records relative to the time of bacteriologic culture.

²Average herd SCC $> 200,000$ cells/mL.

³Average herd SCC $\leq 200,000$ cells/mL.

Table 11. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the diagnostic tests with the geometric mean quarter-composite milk SCC (GEO2¹) as predictor of the cow's infection status when threshold values were set at $\geq 50,000$, 100,000, and $\geq 200,000$ cells/mL stratified by average herd SCC, parity, and stage of lactation

Stratum	No.	Quarter-cSCC	Cows infected by major pathogen								
			Se		Sp		PPV		NPV		
			%	95% CI	%	95% CI	%	95% CI	%	95% CI	
All cows	195										
		≥50,000	95.3	89.1–100	33.6	26.0–41.1	28.9	21.4–36.3	96.2	91.1–100	
		≥100,000	86.0	75.6–96.4	53.9	46.0–61.8	34.5	25.5–43.5	93.1	87.9–98.4	
		≥200,000	65.1	50.9–79.4	76.3	69.6–83.1	43.8	31.6–55.9	88.5	83.1–94.0	
Average herd SCC	120										
High-prevalence herd ²		≥50,000	97.4	92.5–100	21.0	12.1–29.9	37.3	27.9–46.6	94.4	83.9–100	
		≥100,000	89.7	80.2–99.2	41.9	31.2–52.7	42.6	31.9–53.3	89.4	79.7–99.2	
		≥200,000	66.7	51.9–81.5	63.0	52.4–73.5	46.4	33.4–59.5	79.7	69.8–89.5	
Low-prevalence herd ³	75										
		≥50,000	75.0	32.6–100	47.9	36.3–59.5	7.5	0.0–15.7	97.1	91.6–100	
		≥100,000	50.0	1.0–99.0	67.6	56.7–78.4	8.00	0.0–18.6	96.0	90.5–100	
		≥200,000	50.0	1.0–99.0	91.5	85.1–98.0	25.0	0.0–55.0	97.0	92.9–100	
Parity	58										
Heifers		≥50,000	87.5	64.6–100	44.0	30.2–57.8	20.0	6.7–33.3	95.7	87.3–100	
		≥100,000	75.0	44.9–100	66.0	52.8–79.1	26.0	8.1–44.0	94.2	86.6–100	
		≥200,000	37.5	4.0–71.0	80.0	68.9–91.1	23.1	2.0–46.0	88.9	79.7–98.1	
Multiparous	137										
		≥50,000	97.1	91.6–100	28.4	19.7–37.2	31.8	23.0–40.6	96.7	90.2–100	
		≥100,000	88.5	78.0–99.1	48.0	38.3–57.7	36.9	26.5–47.2	92.4	85.3–99.5	
		≥200,000	71.4	56.5–86.4	74.5	66.1–83.0	49.0	35.3–62.7	88.4	81.6–95.1	
DIM	15										
≤100		≥50,000	100	100–100	35.7	10.6–60.8	10.0	0.0–28.6	100	100–100	
		≥100,000	100	100–100	71.4	47.7–95.0	20.0	0.0–55.0	100	100–100	
		≥200,000	100	100–100	78.6	57.1–100	25.0	0.0–67.4	100	100–100	
>100 and ≤199	57										
		≥50,000	83.3	62.2–100	48.9	34.3–63.5	30.3	14.6–46.0	91.7	80.6–100	
		≥100,000	66.6	39.9–93.3	73.3	60.4–86.2	40.0	18.5–61.4	89.1	79.1–99.1	
		≥200,000	58.3	30.4–86.2	86.7	76.7–96.6	53.8	26.7–80.9	88.6	79.3–98.0	
≥200	123										
		≥50,000	100	100–100	26.0	16.9–34.7	30.0	21.3–39.4	100	100–100	
		≥100,000	93.3	84.4–100	41.9	31.9–51.9	34.1	23.8–44.4	95.1	88.5–100	
		≥200,000	66.7	49.8–83.5	71.0	61.7–80.2	43.0	28.4–56.7	87.0	79.2–94.4	

¹Test was conducted for the geometric mean of the last 2 quarter-cSCC records relative to the time of bacteriologic culture.

²Average herd SCC $> 200,000$ cells/mL.

³Average herd SCC $\leq 200,000$ cells/mL.

ing optimal SCC thresholds have been conducted in a very small number of nonrandomly selected and often noncommercial dairy farms (Dohoo and Leslie, 1991; Schepers et al., 1997; Sargeant et al., 2001), limiting the ability to generalize the calculated thresholds to other herds.

Only cows with at least 4 quarter-cSCC records before sampling for bacteriologic culture were included in the final data set, limiting the number of total animals and number of animals per herd that could be taken into account for the different test characteristics. Including all cows that were sampled for bacteriologic culture and had at least 1 quarter-cSCC record around bacteriologic culture would have resulted in a larger data set and more cows per herd and probably in a more precise and accurate calculation of the different test characteristics, particularly those for the last quarter-cSCC record. Also, a more accurate comparison of the effect of factors such as parity, DIM, and herd milk SCC with smaller 95% CI would have been obtained. In that case, however, direct comparison of the different test characteristics for those based on a single observation and those based on a geometric mean of the last 2 or 3 test-day records would not have been possible.

Direct comparison of Se and Sp estimates among different studies is difficult due to differences in definition of IMI status and in distribution of mastitis pathogens. Minor pathogens generally induce a smaller SCC response than major pathogens, inducing a higher number of false-negative results independent of the selected threshold. Sensitivity increased in this study, with almost 20% at a threshold of 200,000 cells/mL, when only cows who were culture-positive for a major pathogen were considered infected, but the PPV decreased to almost 50%. This latter finding might be at least partly explained by the lower prevalence of IMI caused by major pathogens compared with that of IMI caused by minor pathogens.

Calculating the PPV to differentiate IMI with major pathogens from uninfected animals resulted in an enormous increase in the PPV value (data not shown). Interestingly, using a threshold of 200,000 cells/mL, we obtained Sp values comparable to the studies of Schepers et al. (1997) and Dohoo and Leslie (1991), in which a quarter was considered infected if the same mastitis pathogen was isolated from duplicate milk samples or at least 2 out of 3 consecutive samples at a 1-wk interval, respectively. The latter finding indicates that our results were probably only slightly affected by the usually inferior Se of bacteriologic culture using single quarter milk samples (i.e., some of the quarters in our study might have been considered uninfected even though they were infected and exceeded the selected SCC threshold).

The substantially higher Sp for IMI with major pathogens in LP herds compared with HP herds at a threshold of 200,000 cells/mL might be explained by a higher prevalence of chronically infected cows in HP herds than in LP herds. The well-known intermittent shedding of *Staph. aureus* in milk potentially increases the risk of having a culture-negative milk sample from infected cows with a high quarter-cSCC, which obviously leads into more false-positive results (Melchior et al., 2006; Boonyayatra et al., 2014). Although Sp was lower for IMI with a major pathogen in HP herds than in LP herds, the PPV in HP herds was larger than that in LP herds, reinforcing the major effect of the prevalence of a disease on the PPV of a diagnostic parameter.

Stratification by parity showed lower Se but higher Sp for heifers compared with multiparous cows for both IMI with any pathogen and IMI with major pathogens. This finding corresponded well with results obtained by Laevens et al. (1997), in which a lower increase in SCC was observed in infected versus uninfected heifers than in infected versus uninfected second and third lactation cows. The higher PPV in multiparous cows compared with heifers, particularly for IMI with a major pathogen, was probably also driven by the higher prevalence of IMI with a major pathogen in adult cows than in heifers. Multiparous cows are in general more susceptible to IMI than heifers. On 396 Dutch dairy farms, van den Borne et al. (2010) observed an approximately 2 times higher proportion of multiparous cows with a quarter-cSCC higher than 200,000 cells/mL during lactation than heifers.

CONCLUSIONS

Composite milk samples are still widely used as an indicator of subclinical mastitis in dairy cattle. The data suggest using a higher threshold level if one is primarily interested in cows infected with major pathogens. The lower PPV estimates for cows infected with major pathogens compared with those for cows infected with any pathogen can most probably be attributed to the lower prevalence of IMI with major pathogens. We observed no major differences were observed between estimates of the test characteristics and predictive values of the quarter-cSCC criteria based on a single observation and the geometric mean of multiple observations. The results of this study suggest that the quarter-cSCC criterion and SCC threshold value for selecting cows with subclinical mastitis for bacteriologic culture should be tailored to the pathogen group of interest, the herd prevalence of IMI, and the parity of the cows to improve quarter-cSCC as a predictor of IMI.

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