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Evaluation of somatic cell count thresholds to detect subclinical mastitis in Gyr cows

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ABSTRACT

The objectives of this study were (1) to determine the sensitivity (Se) and specificity (Sp) of somatic cell count (SCC) thresholds to identify subclinical mastitis in Gyr cows caused by major and minor pathogens; (2) to study the effects of month of sampling, rear or front mammary quarters, herd, intramammary infection (IMI), and bacterial species on SCC at quarter level; and (3) to describe the prevalence of IMI in Gyr cows in commercial dairy herds. In total, 221 lactating Gyr cows from 3 commercial dairy farms were selected. Milk samples were collected from individual quarters once a month for 1 yr from all lactating cows for SCC and bacteriological analysis. Mammary quarters were considered the experimental units and the SCC results were $\log_{10}$-transformed. Four SCC thresholds (100, 200, 300 and 400 $\times$ 10^3 cells/mL) were used to determine Se and Sp to identify infected mammary quarters. The overall prevalence of IMI in quarter milk samples of Gyr cows was 49.8%, and the prevalence of minor pathogens was higher (31.9%) than that of major pathogens (17.8%). Quarter samples with microbial isolation presented higher SCC compared with negative samples. Sensitivity and Sp of selected SCC thresholds varied according to the group of pathogen (major and minor) involved in the IMI definition. Sensitivity increased and Sp decreased when mammary quarters with only major pathogens isolation were considered positive. The use of a single SCC analysis to classify quarters as uninfected or infected in Gyr cows may not be a useful test for this breed because Se and Sp of SCC at the studied thresholds were low. The occurrence of IMI and the bacterial species are the main factors responsible for SCC variation in mammary quarters of Gyr cows. Milk samples with major pathogens isolation elicited higher SCC than those with minor pathogens.

Key words: intramammary infection, bacteria, sensitivity, specificity

INTRODUCTION

Mastitis is the most common disease in lactating cows and it causes great economic loss to the dairy industry (Halasa et al., 2007). Mastitis is defined as a mammary gland inflammation generally caused by bacterial infection (Santos et al., 2003). During a mastitis episode, defense cells migrate from the blood to the mammary gland to combat the infectious agents, which increases milk SCC (Rainard and Riollet, 2006).

It is essential to monitor IMI to maintain milk quality and herd health. Several methods exist for diagnosing mastitis, and bacteriologic culture of milk samples is considered the standard method (Dohoo et al., 2011), but it is generally expensive and time-consuming for routine screening. Consequently, other diagnostic methods, such as individual SCC, are currently used to screen for IMI status at the herd level, because SCC is widely available to dairy farmers (Schukken et al., 2003).

Somatic cell count is used worldwide as an indicator of subclinical mastitis (Laevens et al., 1997), and it is an important test to assess the efficiency of a mastitis control program in dairy herds (Schukken et al., 2003). One SCC application involves the classification of mammary quarters or cows as infected or uninfected (Dohoo and Leslie, 1991). Many factors may affect SCC such as age, lactation period, parity, season, stress, management, day-to-day variation, and mainly the IMI status (Sargeant et al., 2001; Pyörälä, 2003; Berglund et al., 2007).

Somatic cell count can also be used to estimate the likelihood that a cow or quarter is infected. For this purpose, it is necessary to establish SCC thresholds to classify infected and uninfected cows, especially when the objective is to adopt mastitis prevention and control practices (Schukken et al., 2003). Generally, values of 100 $\times$ 10^3 (Schwarz et al., 2010), 250 $\times$ 10^3 (Berry and Meaney, 2006) or 300 $\times$ 10^3 cells/mL (Deluyker et al., 2005) have been used as the limit to differentiate infected mammary quarters from uninfected, and to decrease the occurrence of false-negative and false-positive results. Using an SCC threshold of 200 $\times$ 10^3 cells/mL, Schepers et al. (1997) and Dohoo and Leslie

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(1991), respectively, found sensitivity (**Se**) of 74 and 83% and specificity (**Sp**) of 89 and 53%, respectively, in Holstein cows.

In Brazil, most dairy herds are composed of Gyr cows and crossbred animals (Holstein × Gyr) (Fonseca et al., 2009). Despite having lower milk yield compared with Holstein cows, Gyr cows have been extensively used either as pure breed or crossbred for milk production in tropical conditions, mainly because animals are well adapted to this environment and are resistant to some tropical diseases (Fonseca et al., 2009). Considering the high prevalence of subclinical mastitis in Gyr cows (Porcionato et al., 2010), the use of SCC as a screening tool to detect subclinical IMI at the cow level would be important for dairy farmers and veterinarians. However, the Se and Sp of SCC thresholds to detect subclinical IMI in Gyr cows have not been determined.

Our objectives were (1) to determine the Se and Sp of SCC thresholds to identify subclinical mastitis caused by major and minor pathogens in Gyr cows; (2) to study the effects of month of sampling, mammary quarter location (rear or front), herd, IMI, and bacterial species on SCC at the quarter level; and (3) to describe the prevalence of IMI in Gyr cows in commercial dairy herds.

MATERIALS AND METHODS

Herd and Animal Selection

In total, 221 lactating purebred Gyr cows from 3 commercial dairy farms (A, B, and C) located in states of São Paulo and Minas Gerais, Brazil, were selected on the basis of their proximity to our laboratory. Quarter milk samples were obtained on from 1 to 8 occasions from 221 lactating cows, resulting in a total of 2,836 samples collected monthly at morning milking during 1 yr (July 2008 to June 2009). The samples were collected from all lactating cows at the time of farm visits, except those with clinical mastitis symptoms. Cows exhibiting symptoms of clinical mastitis were not recorded.

All dairy cows were milked twice daily and, based on a limited number of observations, lactating cows had estimated milk yield of 14 kg/d, lactation length of 8 mo, and parity number from 1 to 6. Cows were handled on tropical pasture system, with free access to water, and were fed a concentrate supplement after milking, according to milk yield and stage of lactation. Calvings were assumed to occur randomly over the year, but no calving dates were recorded.

In herd A, hand-milking was used, and no mastitis control practices were adopted at milking (except for dry cow therapy and clinical mastitis treatment). Herd B used mechanic milking in a double-4 herringbone milking parlor and adopted the following mastitis control practices at milking: forestripping in a strip cup to check for clinical mastitis, predipping, and drying teats with disposable towels. Dry cow therapy and clinical mastitis treatment were also adopted. Cows from herd C were mechanically milked in a double-6 tandem milking parlor with the same mastitis control program practices as those adopted in herd B. In all 3 herds, calf suckling before milking was necessary to stimulate milk let-down in dairy cows. In all selected herds, postdipping was not used because calves had access to mammary glands for suckling at the end of each milking.

Sampling and Analysis

Before sampling, teat ends were cleaned with iodine solution (0.5%), allowing approximately 20 s of contact time before wiping the teats with disposable towels. Each teat end was scrubbed vigorously with a cotton pledget soaked with ethyl alcohol (70%). The first streams of foremilk were discharged, and then 10 mL of milk was collected aseptically from each teat into sterile vials for microbiological culture. Quarter milk samples were collected and preserved with bronopol for SCC analysis. After collection, milk samples were stored at 4°C (maximum time: 48 h) until bacteriological culture and SCC tests were performed. Milk SCC was determined using a flow cytometry method (Somacount 300, Bentley, Chaska, MN).

Bacteriological culture was performed according to National Mastitis Council standards (National Mastitis Council, 1999). Briefly, from each sample, 0.01 mL of milk was plated on blood agar and incubated aerobically for 24 and 48 h at 37°C. A quarter was considered culture-positive when growth of at least one colony was detected on streaks. Samples yielding more than 2 different bacterial species were considered to be contaminated and removed from the statistical analysis. Bacteria were identified based on colony morphology and Gram staining. For gram-positive cocci, catalase tests with hydrogen peroxide (3%) were used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Coagulase test was carried out using sterile rabbit plasma to distinguish *Staphylococcus aureus* (coagulase-positive) from non-aureus staphylococci, referred to as CNS. Streptococci were subdivided into esculin-positive cocci and esculin-negative cocci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*, respectively). The Christie, Atkins, Munch-Petersen (CAMP) test was used to differentiate *Strep. agalactiae* from *Strep. dysgalactiae*. Isolated pathogens were considered as major (*Staph. aureus*, *Streptococcus uberis*, *Strep. agalactiae*, *Strep. dysgalactiae*) and minor (CNS and *Corynebacterium* spp.).

Statistical Analysis

Results of SCC were $\log_{10}$-transformed to obtain a normal distribution. All statistical models were analyzed using PROC MIXED (SAS Institute, 2003). Two data sets were used for statistical analysis; the first (data set 1) comprised all collected samples (positive and negative cultures), and the second (data set 2) consisted of culture-negative samples only. For analysis of data set 1, 3 different models were used ([1], [2], and [3]):

$$Y_{ijklm} = \mu + H_i + C_j(H_i) + M_k + Q_l + IMI_m + e_{ijklm}, \quad [1]$$

$$Y_{ijklm} = \mu + H_i + C_j(H_i) + M_k + Q_l + B_m + e_{ijklm}, \quad [2]$$

$$Y_{ijklm} = \mu + H_i + C_j(H_i) + M_k + Q_l + P_m + e_{ijklm}, \quad [3]$$

where Y is the observed value of log SCC, μ is the overall mean, H_i is the random effect of the herd i ($i = 1$ to 3), $C_j(H_i)$ is the random effect of cow j ($j = 1$ to 221) nested within herd i, M_k is the fixed effect of month of sampling k ($k = 1$ to 12), Q_l is the fixed effect of rear or front mammary quarters l ($l = 1$ to 2), IMI_m is the fixed effect of IMI m (m = presence and absence), B_m is the fixed effect of bacterial species m (m = *Staph. aureus*, *Strep. uberis*, *Strep. agalactiae*, *Strep. dysgalactiae*, CNS, *Corynebacterium* spp., or culture-negative samples), P_m is the fixed effect of the pathogens group m (m = major, minor, or negative samples), and e_{ijklm} is the random error term.

For analysis of data set 2 (only culture negative samples), model [4] was used:

$$Y_{ijkl} = \mu + H_i + C_j(H_i) + M_k + Q_l + e_{ijkl}, \quad [4].$$

where Y is the observed value of log SCC, μ = overall mean, H_i = random effect of the herd ($i = 1$ to 3), $C_j(H_i)$ = random effect of the cow nested within herd ($j = 1$ to 207), M_k = fixed effect of month of sampling ($k = 1$ to 12), Q_l = fixed effect of rear or front mammary quarters ($l = 1$ to 2), e_{ijkl} = random error term.

Means of SCC were computed using the LSMEANS option. Differences among least squares means were tested using the PDIFF option in the LSMEANS statement. For all statistical analyses, significance was declared at $P \leq 0.05$.

Se and Sp of SCC Thresholds

Four SCC thresholds (100, 200, 300, and 400 $\times$ 10^3 cells/mL) were tested to determine the Se, Sp, and predictive value (positive and negative) to correctly identify infected mammary quarters. Bacteriological culture was considered the reference method to evaluate the SCC thresholds.

RESULTS AND DISCUSSION

IMI Prevalence

Bacteriological culture results were obtained from 2,870 quarter milk samples; 34 (1.2%) contaminated samples were not included in the statistical analysis. The overall prevalence of IMI in examined samples was 49.8% of the mammary quarters (Table 1). Considering only positive-culture samples, the bacterial species most frequently isolated were *Corynebacterium* spp. (35.5%), *Staph. aureus* (30.8%), CNS (28.7%), *Strep. uberis* (3.8%), and *Strep. dysgalactiae* (1.3%). Overall prevalence was higher for minor pathogens (64.2%) than for major pathogens (35.8%). Schepers et al. (1997) and Dohoo and Leslie (1991) also reported higher prevalence of minor pathogens (*Corynebacterium* spp. and CNS) compared with major pathogens (*Strep. uberis*, *Strep. dysgalactiae*, and *Staph. aureus*) in Holstein cows. Similar results were found by Souto et al. (2008) in Holstein cows, in which *Corynebacterium* spp. were the most isolated bacteria (11.2%), followed by *Staphylococcus* spp. (9.2%) and S*treptococcus* spp. (5.3%). The absence of postdipping procedures may explain the high IMI prevalence caused by *Corynebacterium* spp. in this study (Hillerton et al., 1995).

The prevalence of pathogen groups varied among herds (Table 1). For example, minor pathogens were predominantly found in herds A and C. In herd A, CNS (39.0%) and *Corynebacterium* spp. (39.5%) were the most prevalent bacterial species isolated. In herd B, *Staph. aureus* (54.9%) and *Corynebacterium* spp. (27.3%) were the predominant isolates. In herd C, CNS (33.1%) and *Corynebacterium* spp. (39.27%) were predominant. These differences in mastitis pathogen prevalence among herds can be explained by different hygiene and management procedures used in each herd. The higher prevalence of IMI in studied herds indicates that standard mastitis prevention programs have not been successfully implemented.

Overall prevalence of *Staph. aureus* was high in studied herds (30.8%), which suggests that the prevention of spread of contagious bacteria during milking was not effective. Postdipping is usually not adopted in Gyr herds because calves stay with cows after milking to suck the residual milk. The large variation in prevalence of *Staph. aureus* among herds may be explained by differences in milking routines, milking equipment maintenance, and strategies used to segregate or to cull *Staph. aureus*-infected cows (Piepers et al., 2007).

Table 1. Prevalence of mastitis pathogens in quarter milk samples in Gyr cows from 3 herds

Microorganism	Herd (n)				All quarters (%)	Culture-positive (%)
	A	B	C	Total		
Quarters analyzed bacteriologically, no.	1,210	737	889	2,836		
Culture-negative quarters, no.	772	265	388	1,425	50.2	
Culture-positive quarters, no.	438	472	501	1,411	49.8	
Staphylococcus aureus	66	259	109	434	15.3	30.8
Streptococcus uberis	17	11	25	53	1.9	3.8
Streptococcus dysgalactiae	11	5	2	18	0.6	1.3
CNS	171	68	166	405	14.3	28.7
Corynebacterium spp.	173	129	199	501	17.7	35.5
Contamination, no.	13	9	12	34	1.2	2.4
Pathogen group,[1] no.						
Major pathogens	94	275	136	505	17.8	35.8
Minor pathogens	344	197	365	906	31.9	64.2

[1]Major pathogens = *Staph. aureus*, *Strep. uberis*, and *Strep. dysgalactiae*; minor pathogens = CNS, *Corynebacterium* spp.

Factors Affecting SCC

Data set 1 included 2,836 observations from 221 cows in 3 herds. The overall mean log SCC of all mammary quarters (infected and uninfected) was 5.08 ± 0.82 (120,226 cells/mL). All effects included in the 3 models used to analyze data set 1 were significant. The occurrence of IMI ($P = 0.001$), microorganism species ($P = 0.001$), and pathogen type ($P = 0.001$) had the highest F-values in models [1], [2], and [3], respectively, indicating that the presence of IMI and type of microorganism involved were the principal factors responsible for SCC variation, which is in agreement with Harmon (1994). Month of sampling had a significant effect on SCC in all studied models used for analysis of data set 1; however, this effect explained only a small proportion of SCC variation, as reported by Harmon (1994). The lowest SCC value was 49,000 cells/mL in October compared with a high SCC of 436,000 cells/mL in June; however, no difference was found between the summer (October to March, average 123,000 cells/mL) and winter months (April to September, average 117,000 cells/mL). These results were unexpected because seasonal variation in individual cow SCC has been reported in herds with year-round calving with the highest SCC values in the summer (Green et al., 2006).

Although the effect of mammary quarter location (rear versus front) was significant, it had less influence on log SCC ($P = 0.009$) than IMI. In our study, rear mammary quarters had higher log SCC (5.10) compared with front quarters (5.03); however, prevalence of IMI in front (50.5%) and rear quarters (48.9%) did not differ. Schepers et al. (1997) reported a higher prevalence of IMI in rear quarters and a higher SCC in rear quarters than in front quarters. Similar to our results, results of Schwarz et al. (2010) showed a higher log SCC (4.84) in rear quarters compared with front quarters (4.81), but IMI prevalence among rear and front quarters could not explain the difference in SCC.

Results of average log SCC distributed according to the microorganism involved in IMI are presented in Table 2. Infected quarters had higher log SCC (5.32) compared with uninfected ones (4.9). Schepers et al. (1997) also found a significant effect for the presence of IMI on log SCC, with higher SCC for infected mammary quarters. Ogola et al. (2007) reported that culture-positive samples showed higher log SCC (5.79) than culture-negative samples (5.42). The log of SCC was higher in samples with isolation of major pathogens (5.51) compared with those with minor pathogens (5.31). Our results are similar to those reported by Djabri et al. (2002), because quarters infected by minor pathogens had a log SCC between 5.04 and 5.17, and quarters infected by major pathogens had a log SCC >5.51. These results indicate that each mastitis-causing pathogen was able to stimulate different magnitudes of immune response in host animal. The magnitude of cellular response, the duration of infection, and the animal's exposure to previous mastitis may influence SCC results.

Considering only culture-negative samples (data set 2), the overall mean log SCC was 4.85 ± 0.80 (70,794 cells/mL) for 1,425 observations, which is similar to the average SCC of quarters with no IMI (75,000 cells/mL) reported by Olde Riekerink et al. (2007). In a meta-analysis study, Djabri et al. (2002) estimated that the geometric mean of SCC in bacteriologically negative quarters was 68,000 cells/mL. In our study, in the culture-negative data set, location of mammary quarter ($P = 0.36$) had no effect on log SCC; however, month of sampling was the principal factor responsible for the SCC variation ($P < 0.001$).

Se and Sp of SCC Thresholds

Sensitivity, Sp, positive predictive value (**PPV**), and negative predictive value of selected SCC thresholds to identify subclinical infections caused by major and

Table 2. Log SCC means of mammary quarters, according to the microorganism species and pathogen group as the cause of intramammary infection in Gyr cows

Microorganism	No.	Log SCC (cells/mL)	SEM
Streptococcus dysgalactiae	18	5.98[a]	0.21
Staphylococcus aureus	434	5.94[a]	0.03
Streptococcus uberis	53	5.52[b]	0.09
CNS	405	5.34[c]	0.03
Corynebacterium spp.	501	5.29[c]	0.03
Culture-negative samples	1,425	4.93[d]	0.02
Contamination	34		
Pathogen group			
Major pathogens[1]	505	5.51[a]	0.03
Minor pathogens	906	5.31[b]	0.02

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Major pathogens = *Staph. aureus*, *Strep. uberis*, and *Strep. dysgalactiae*; minor pathogens = CNS, *Corynebacterium* spp.

minor pathogens in Gyr cows are presented in Table 3. Sensitivity and Sp results of selected SCC thresholds are expressed according to the group (major and minor) of pathogen involved in the IMI definition. When only mammary quarters with major pathogen isolation were considered positive, Se values for all SCC thresholds increased compared with when both major and minor pathogens were considered positive. For example, for the 100×10^3 cells/mL threshold, Se ranged from 66.4% (IMI: major and minor pathogens) to 74.0% (IMI: only major pathogens). Inclusion of minor pathogens as a cause of IMI may increase the number of false-negative results, because this group of pathogens have limited pathological significance, induce a relatively small cell count response, and seldom cause inflammation (Dohoo and Leslie, 1991). The high prevalence of minor pathogens found in our study may justify the low values of Se and Sp.

Using the 200×10^3 cells/mL threshold, Schepers et al. (1997) described Se of 74.56% and Sp of 89.6%, considering that a quarter had an IMI if the same mastitis pathogen was isolated from duplicate foremilk samples collected at a 1-wk interval. Schepers et al. (1997) reported higher Se and Sp values for all studied thresholds compared with our study. For example, for the 100×10^3 cells/mL threshold, reported Se and Sp were, respectively, 83.2 and 80.5%. However, the PPV described by Schepers et al. (1997) were lower (maximum value 26.9%) compared with those found in our study (maximum value 65.9%), because Schepers et

Table 3. Sensitivity, specificity, and predictive value (positive and negative) of 4 SCC thresholds (100 to 400 $\times 10^3$ cells/mL) to identify IMI, considering 3 criteria for IMI in mammary quarters of Gyr cows

Criteria and SCC threshold	Sensitivity	Specificity	Predictive value,[1] % (95% CI)	
			PPV	NPV
IMI = major and minor pathogens; UMQ[2] = only culture-negative samples				
100	66.4 (63.9, 68.8)	59.1 (56.5, 61.6)	61.6 (59.1, 64.0)	64.1 (61.4, 66.6)
200	51.5 (48.9, 54.2)	71.8 (69.4, 74.2)	64.4 (61.5, 67.2)	60.0 (57.7, 62.4)
300	43.2 (40.6, 45.8)	77.4 (75.2, 79.6)	65.4 (62.2, 68.4)	58.0 (55.7, 60.2)
400	37.1 (34.6, 39.7)	81.2 (79.0, 83.1)	66.0 (62.6, 69.3)	56.6 (54.5, 58.8)
IMI = only major pathogens; UMQ = minor pathogens and culture-negative samples				
100	74.0 (69.9, 77.7)	50.8 (48.8, 52.9)	24.5 (22.4, 26.8)	90.0 (88.3, 91.6)
200	59.3 (54.9, 63.6)	64.4 (62.4, 66.4)	26.5 (23.9, 29.1)	88.0 (86.3, 89.5)
300	51.0 (46.5, 55.4)	71.1 (69.2, 72.9)	27.6 (24.8, 30.6)	87.0 (85.4, 88.5)
400	45.4 (41.0, 49.1)	75.9 (74.1, 77.6)	28.9 (25.8, 32.2)	86.5 (85.0, 88.0)
IMI = only major pathogens; UMQ = only culture-negative samples				
100	74.0 (69.9, 77.7)	59.1 (56.5, 61.7)	39.0 (35.9, 42.1)	86.6 (84.2, 88.6)
200	59.3 (54.9, 63.6)	71.8 (69.4, 74.1)	42.6 (39.0, 46.4)	83.3 (81.1, 85.4)
300	51.0 (46.5, 55.4)	77.4 (75.2, 79.6)	44.4 (40.3, 48.5)	81.7 (79.5, 83.7)
400	45.4 (41.0, 49.9)	81.2 (79.0, 83.1)	46.0 (41.5, 50.5)	80.8 (78.7, 82.8)

[1]PPV = positive predictive value; NPV = negative predictive value.

[2]UMQ = uninfected mammary quarters.

al. (1997) found lower prevalence of major pathogens compared with our study.

In our study, estimates of Sp ranged from 50.8 to 81.2%, depending on the IMI definition used and on the SCC threshold. Increasing the SCC threshold from 100 to 400 $\times$ 10^3 cells/mL resulted in increased Sp for all IMI definitions. For culture-negative mammary quarters, as the definition for healthy quarters and using 100 $\times$ 10^3 cells/mL threshold, the probability that they would be truly negative was 64.1%. The high PPV (61.4 to 65.3%) for the first IMI (infected quarters having major or minor pathogens isolated) observed in this study was probably because of the high prevalence of IMI (49.8%), because the PPV is influenced by prevalence of the disease (McDermott et al., 1982). Therefore, the use of a single SCC analysis to classify quarters as uninfected or infected may not be a useful for Gyr cows because Se and Sp of SCC at the studied thresholds were relatively low.

According to McDermott et al. (1982), the main objective of choosing a specific SCC threshold is to optimize the efficacy of a mastitis control program. Thus, it is necessary to choose a SCC threshold to minimize IMI diagnostic errors, decrease the spread of infection within a herd, reduce costs with treatment, and to reduce mastitis culling rates. As expected, using the 200 $\times$ 10^3 cells/mL compared with the 100 $\times$ 10^3 cells/mL limit would result in higher probability of the occurrence of false-negative results, which may be critical for the implementation of mastitis control program in Gyr herds, because some infected cows with low SCC could not be correctly identified as a source of infection. Therefore, considering the use of SCC for screening the occurrence of IMI in Gyr cows in herds with high prevalence of both major and minor pathogens, based on the findings of our study, the 100 $\times$ 10^3 cells/mL threshold would be appropriated to differentiate infected from uninfected mammary quarters and to reduce occurrence of false-negative results. These results are in agreement with the recommendations of Pyörälä (2003), which suggested that a culture-negative mammary quarter contains fewer than 100 $\times$ 10^3 cells/mL and that a quarter SCC with >200 $\times$ 10^3 cells/mL is very likely to be infected. Additionally, Schwarz et al. (2010) indicated that quarter SCC >100 $\times$ 10^3 cells/mL are generally related to an inflammatory process in the mammary glands of Holstein cows.

Although bacteriologic culture is considered a reliable method for detection of IMI, many factors can influence the results of bacteriological culture, such as microorganism species, method of sampling, storage condition after collection, number of organisms in each sample, duration of infection, shedding pattern of the organism, medium used for culture, volume of milk plated, and frequency of sampling (Dohoo et al., 2011). Considering that no perfect diagnostic test for IMI is available, culturing triplicate milk samples may be considered the gold standard for IMI detection (Andersen et al., 2010). Therefore, the results of the present study should be interpreted with caution given that milk samples were obtained from a limited number of herds (n = 3) and that IMI definition was based only on culture results of a single foremilk sample. The use of a single milk sample culture is acceptable for most pathogens to identify an IMI with isolation of at least a single colony (from a 0.01-mL milk sample) and resulted in Se of 85.8% and Sp of 75.1% compared with the gold standard of culturing triplicate milk samples (Dohoo et al., 2011). The Se and Sp results of our study may be underestimated for some pathogens, such as *Corynebacterium* spp. and CNS, because the IMI definition was based on a single foremilk sample culture and because of the high prevalence of minor pathogens.

CONCLUSIONS

The use of a single SCC analysis to classify quarters as uninfected or infected may not be a useful test for Gyr cows because the Se and Sp of SCC at the studied thresholds were relatively low. Sensitivity and Sp of selected SCC thresholds varies according to the group of pathogen (major and minor) involved in the IMI definition. Sensitivity is increased and Sp is decreased when only mammary quarters with major pathogens are considered positive. The occurrence of IMI and the bacterial species are the main factors responsible for SCC variation in mammary quarters of Gyr cows. Milk samples with major pathogens have higher SCC compared with those with minor pathogens isolated. Overall prevalence of IMI in Gyr cows was high, and the prevalence of minor pathogens was higher than that of major pathogens.

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