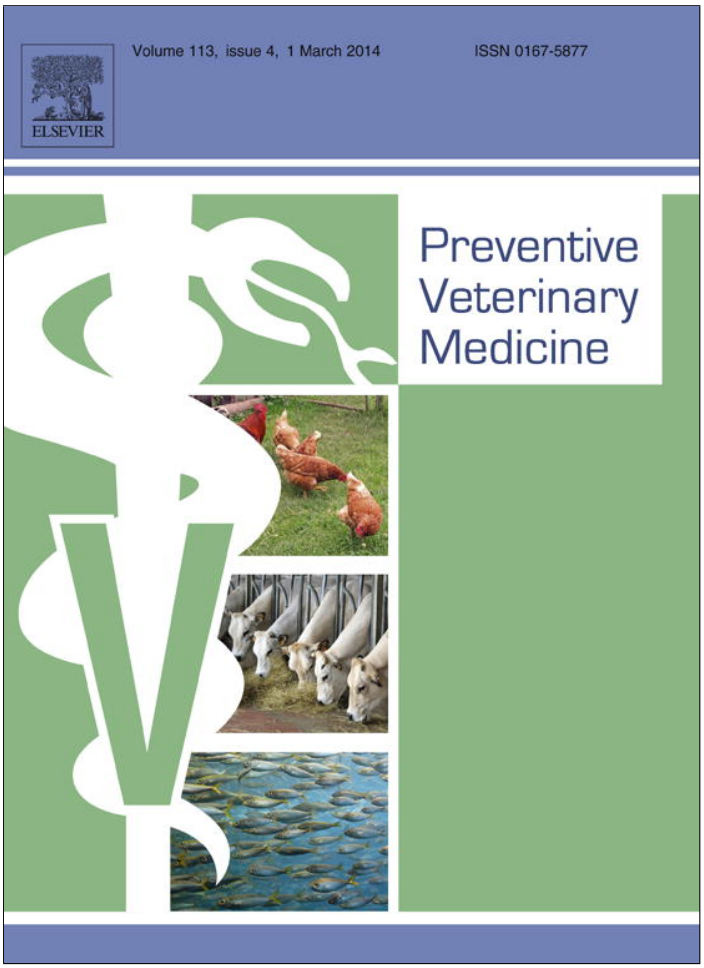

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Accuracy of the composite somatic cell count to detect intra-mammary infection in dairy cows using latent class analysis

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ABSTRACT

The somatic cell count (SCC) is considered an important indicator of intra-mammary infection (IMI). The purpose of this study was to determine the accuracy of both SCC and culture to detect IMI and their conditional dependence by means of latent class methods. This study involved 175 dairy cows from 2 herds with different udder infection prevalences. Quarter and composite milk samples were collected for SCC and bacteriological culture. Latent-class models using Bayesian methods were used to estimate test sensitivity (Se) and specificity (Sp) and population prevalence. The models ran involved only major mastitis pathogens and composite SCC (CSCC). Five thresholds between 100,000 and 300,000 cells/mL were evaluated and the receiver operating characteristics (ROC) curve analysis was performed. Fifty-five percent of the cows had CSCC ≥200,000 cells/mL and 95.4% of the cows had at least one infected quarter either with minor or major pathogens. Considering a threshold of 150,000 cells/mL, the estimated Se and Sp for the CSCC were, 0.80 (95% CrI 0.71–0.88) and 0.57 (95% CrI 0.44–0.71), respectively. The estimated culture Se and Sp were 0.83 (95% CrI 0.73–0.93) and 0.89 (95% CrI 0.74–0.98), respectively. There was no evidence of dependence between CSCC and culture. The area under curve for CSCC was 0.72. To the best of our knowledge, this is the first report of the CSCC accuracy to detect IMI for major pathogens considering the effect of culture misclassification. The estimates provided here could help to examine the performance of sampling schemes based on CSCC to manage udder health.

1. Introduction

Worldwide, mastitis is the most important production disease of dairy cattle causing decreased milk yield and lower milk quality (Seegers et al., 2003; Pyörälä, 2003; Halasa et al., 2007). Subclinical mastitis can overall constitute up to 80% of the total losses attributed to the disease and typically manifests as an elevation in the somatic cell count (SCC) (Halasa et al., 2007).

It is well known that the increasing SCC in milk is a manifestation of the inflammatory response to intra-mammary infection (IMI) (Schukken et al., 2003). Thus, SCC is recognized as an indirect measure of IMI (Holdaway et al., 1996). As a herd management tool, SCC can be performed at quarter and cow levels. Quarter samples are more suitable than composite samples to assess the association between IMI

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and SCC (Schukken et al., 2003). In contrast, the somatic cell counting using composite samples is less time consuming and less costly (Sargeant et al., 2001; Pyörälä, 2003; Schukken et al., 2003).

Herd decisions based on diagnostic tools, require the awareness about test accuracy. In this regard, SCC sensitivity (Se) and specificity (Sp) have been previously assessed using microbiology culture as the gold standard, both at quarter (Timms and Schultz, 1987; Schepers et al., 1997) and cow level (McDermott et al., 1982; Dohoo and Leslie, 1991). Because bacteriological culture is not a perfect test for IMI diagnosis (Erskine and Eberhart, 1988; Bradley et al., 2005), classification errors in the reference test should be considered to avoid serious bias in the accuracy of the test under evaluation (Enøe et al., 2000). There are different models to account for classification error, the latent class model (Hui and Walter, 1980) allow estimation of the Se and Sp of two tests. This model is based on cross-classified results, when applied to individuals from two or more populations with different disease prevalences. The solutions to this problem can be accomplished either by maximum likelihood procedures or by Bayesian methodology (Enøe et al., 2000). The Bayesian approach allows the integration of prior data and/or expert opinion about prevalence and test accuracy (and the uncertainty associated with each value) with the current survey data (usually termed as likelihood) to produce updated posterior inferences. In addition, the correlation between tests can be evaluated fitting a conditional dependence model in a Bayesian framework (Georgiadis et al., 2003).

Sanford et al. (2006) evaluated the accuracy of the California Mastitis Test (CMT) for detecting the presence of IMI in cows using a Bayesian latent class analysis. However, the Se and Sp of milk composite SCC (CSCC) using a Bayesian latent class analysis has not been yet reported. This would be relevant considering the CMT disadvantage, its difficulty to be read due to the subjectivity of the scoring, which might result in false positive and false negative (Viguier et al., 2009).

The present research has been conducted to determine the accuracy of both CSCC and culture to detect IMI and their conditional dependence by means of the latent class method.

2. Materials and methods

2.1. Definition of infection

The latent class models create a probabilistic definition of infection, which is based on the assumption that both tests used contain information about the same latent condition (Enøe et al., 2000). In our case, we assume that this condition is IMI due to major pathogens, which can be defined as the presence of major pathogens in the udder gland. A positive culture indicates the presence of viable bacteria in milk. As result of this, an inflammatory process is triggered in the udder, evidenced by an increasing milk somatic cell count (Schukken et al., 2003), particularly when IMI is caused by major pathogens (Harmon, 1994). The definition of the latent disease is based on both tests because CSCC and culture are combined in the latent class model.

2.2. Study population

A sample of 175 Holstein dairy cows selected at random from 2 dairy herds located in Córdoba, Argentina was used in this study. Herds were selected based on their proximity to the academic institution carrying out the study, willingness of the farmer to cooperate, and availability of animal identification and registrations. In addition, these herds were selected because they showed differences in bulk milk SCC. The sampling fractions in both herds were similar.

2.3. Sample collection

Milk samples were collected following standard procedures (National Mastitis Council, 2004). Pre-milking udder preparation was performed as the farm's usual practice. Teat ends were scrubbed with alcohol and allowed to dry. Foremilk was stripped from each quarter prior to the sampling. Milk samples collected in sterile vials were cooled with ice-packs and immediately transported to the laboratory for further procedures.

2.4. Bacteriology and somatic cell count

From each quarter milk sample, 0.01 mL was cultured on Trypticase Soy Agar plates (BBL, Cockeysville, MD, USA) containing 5% sheep blood. The plates were incubated at $37 \pm 1\,^{\circ}C$ for 48 h. The plates were observed for bacterial growth after an incubation period of 24 and 48 h. Following colony morphology and haemolytic patterns on blood agar observation, isolates were further examined by means of Gram staining of organisms, catalase and oxidase testing and additional biochemical and metabolic tests for major pathogens and coagulase-negative staphylococci (CNS).

A sample was considered positive when growth of $\geq$3 cfu/mL of a particular organism and <3 colony types on the plate was detected. For *Staphylococcus* spp., a minimum of 1 cfu was required. Samples yielding >3 colony types were considered to be contaminated and were excluded from the analysis.

The bacterial isolates were stored at $-20\,^{\circ}C$ in tryptic soy broth containing 15% of glycerol. Streptococci, enterococci and enterobacteria were identified using methodology based on National Mastitis Council (2004) standards. Colonies with typical zones of complete and incomplete hemolysis and nonhemolytic colonies that had a positive tube test for free coagulase, aerobic acid production from maltose, positive Voges Proskauer reaction, and growth at $45\,^{\circ}C$ were classified as *Staphylococcus aureus*; the strains that were negative coagulase test and susceptible to the furazolidone test were identified as CNS. All bacteriological analysis were done by the same person, who was blinded with respect CSCC test results.

Bacteriological causes of infection were categorized as major pathogens (*Escherichia coli*, *Klebsiella* spp., environmental (non-agalactiae) streptococci, *S. aureus*, *Streptococcus agalactiae*, and other) or minor pathogens

(coagulase-negative staphylococci and *Corynebacterium bovis*). A quarter was considered infected when at least one major pathogen was isolated, regardless the presence of minor pathogens. The cow was the unit of analysis, therefore, a cow was defined as infected, if at least one quarter was infected (parallel interpretation). A cow with negative cultures in all quarters, was defined as uninfected.

The SCC was performed with a Somacount 300 (Bentley, USA 1997) according to the revised protocol of 148A method C, fluoro-opto-electronic (International Dairy Federation, 1995).

2.5. Statistical analysis

2.5.1. Model and assumptions

We applied latent-class model (Hui and Walter, 1980; Walter and Irwig, 1988; Enøe et al., 2000) in a Bayesian framework (Branscum et al., 2005; Johnson et al., 2001) using a Gibbs sampler to estimate test parameters: Se CSCC (Se_CSCC), Sp CSCC (Sp_CSCC), Se culture (Se_C) and Sp culture (Sp_C) and population prevalences (Spiegelhalter et al., 2004). We assumed that both tests are conditionally independent given disease status, because the tests rely on different biological principles (Georgiadis et al., 2003).

Three assumptions need to be met to validate the method: the diagnostic tests should be conditionally independent of each other, two populations with different prevalences are required and each test must have the same Se and Sp across populations. These assumptions were evaluated as follows:

The assumption about conditional independence between test was evaluated considering a dependence model as described by Georgiadis et al. (2003) and Branscum et al. (2005). The correlation coefficients and the corresponding 95% credible intervals (CrI) were estimated under the dependence model. The estimated CrI included zero and as consequence of that the null hypothesis of conditional independence was not rejected (Gardner et al., 2000). Additionally, Deviance Information Criterion (DIC) was estimated for dependence and independence models. The difference in the DIC of the two models was <2, suggesting that both models fitted the data equally well (Spiegelhalter et al., 2002). Therefore and according to Kostoulas et al. (2006) the independence model was used in the analysis.

The assumption about difference in prevalences between population was met choosing 2 herds with different udder infection status. The udder infection level was inferred considering bulk milk SCC during six consecutive months prior to the study. The median bulk milk SCC was 191,400 cells/mL (standard deviation = 24,700) and 511,800 cells/mL (standard deviation = 127,700) for Herds 1 and 2, respectively.

The assumption about constant Se and Sp across sub-populations was explored by fitting separate Bayesian latent class models with conditional dependence between CSCC and culture for each herd (Branscum et al., 2005). There was overlap of the CrI of the Ses and Sps estimated in each herds separately and the corresponding intervals obtained when estimated in both herds simultaneously.

2.5.2. Priors

Beta distribution Be(a, b) were used as priors for the parameters of interest (Ses, Sps and prevalences). The same priors were used for these parameters for both conditional dependence and independence models (Kostoulas et al., 2006).

The prior distribution for Se_C and Sp_C were based on the study of Sanford et al. (2006), combined with experience gained from diagnostic work. The value of Se_C was determined to be 0.59, while we were 95% sure that it was more than 0.30. The Be with mode 0.59 and 5th percentile 0.30 is Be(5.0274, 3.7987) (Fig. 1). The value of Sp_C was 0.92, and it was thought to be at least 0.60 with 95% certainty. This corresponded to a Be(7.6419, 1.5776) (Fig. 1).

In addition, the prior distribution for Se_CSCC and Sp_CSCC were based on McDermott et al. (1982) and Dohoo and Leslie (1991) studies. The value of Se_CSCC was determined to be 0.85, while we were 95% sure that it was more than 0.50. The Be with mode 0.85 and 5th percentile 0.50 is Be(6.252, 1.9268) (Fig. 1). The value of Sp_CSCC was 0.67, and it was thought to be at least 0.40 with 95% certainty. This corresponded to a Be(7.2181, 4.0626) (Fig. 1).

For the conditional dependence models the Be prior of Se_C was used for both lambda D+ and gamma D+, and the Be prior of Sp_C was used for both lambda D− and gamma D− (Branscum et al., 2005).

The elicitation of the prior distribution for IMI was derived by consensus on the subjective judgment of dairy practitioners of the region (Spiegelhalter et al., 2004). Beta distributions were determined by specifying the mode and either the 5th or 95th percentile (depending on whether the mode was ≥0.50 or <0.50). Prior beta distribution parameters for high and low prevalence herds were Be(9.6, 3.9) and Be(3.9, 9.6), respectively.

2.5.3. Sensitivity analysis

The influence of prior information on the model parameters estimates was assessed considering four additional different sets of prior information for Ses and Sps (Table 1). The same priors used in the primary analysis (Section 2.5.1) were considered for all prevalences.

2.5.4. Receiver operating characteristics (ROC) curve analysis

In each herd, 2×2 tables were created at a number of thresholds values for the CSCC (with classification as CSCC ≥thresholds or CSCC <thresholds) cross-tabulated with Culture+ and Culture− individuals. Five thresholds between 100,000 and 300,000 cells/mL were evaluated considering papers published in the last 10 years (Schukken et al., 2003; Bansal et al., 2005; Deluyker et al., 2005). After creating all the 2×2 tables, Ses and Sps were estimated using both the independence and dependence models. The potential conditional dependence between CSCC and culture was evaluated as earlier described. No evidence of conditional dependence was found whatsoever.

ROC curve was produced based on the posterior medians of Se_CSCC and Sp_CSCC for each threshold (Nielsen

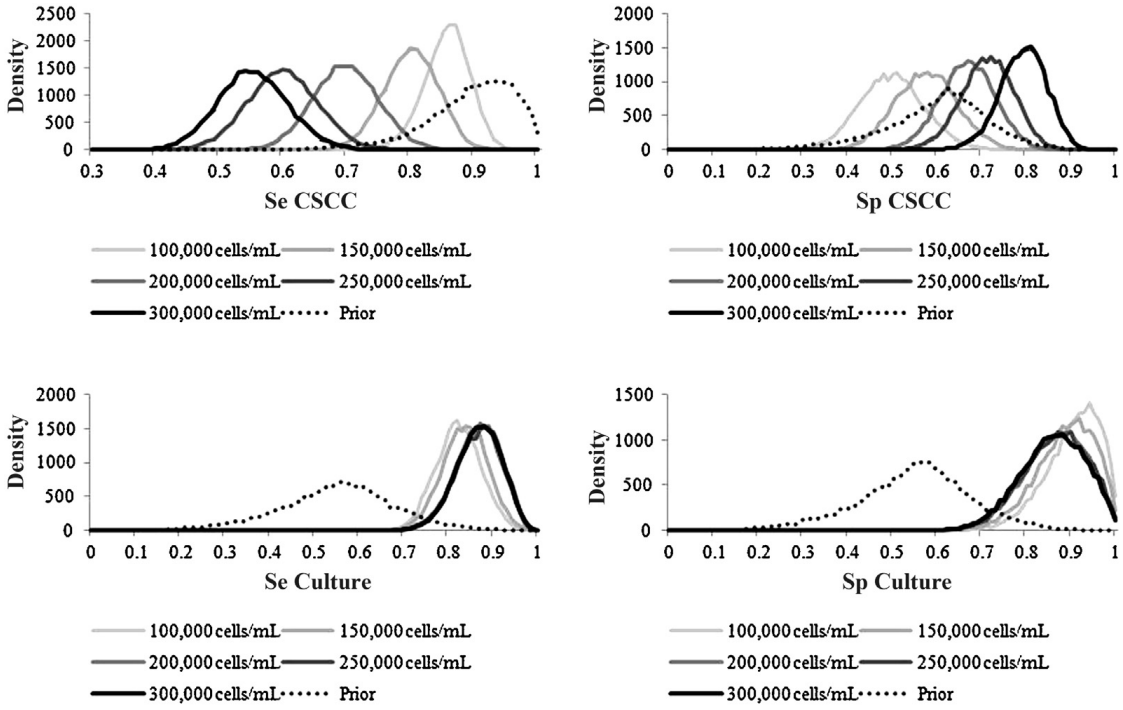

Fig. 1. The Se and Sp prior and posterior density curve for CSCC (above) and culture (below) at five thresholds of CSCC in a study to detect intra-mammary infections in Argentinian dairy herds.

et al., 2002). Subsequently, the point on the ROC curve closest to the upper left corner that optimizes prevalence-independent summary measures of Se and Sp such as the Youden index ($J = Se + Sp\_1$) was selected as a potential cut-off. The area under the curve (AUC) was calculated using "the trapezoidal rule" (Hanley and McNeil, 1983) in order to evaluate the CSCC overall diagnostic performance. To investigate the purpose specific cut-off selection, the constructed curve was used to derive optimal threshold values with regard to a given prevalence and a ratio of the cost of the false positive to the false negative test outcomes. Thresholds evaluation was based on minimization of the misclassification cost term (MCT), which was calculated as:

$$MCT = CFN/CFP * P * (1 - Se\_CSCC) + (1 - P) * (1 - Sp\_CSCC)$$

Table 1
Beta distribution parameters (Be(a, b)) used as priors for Se_CSCC, Sp_CSCC, Se_C and Sp_C.

Set priors	Parameters	Be(a, b)	Mode	95% sure that greater than
I	Se_CSCC	23.9027, 5.0417	0.85	0.70
	Sp_CSCC	91.0649, 45.3603	0.67	0.60
	Se_C	50.687, 35.5283	0.59	0.50
	Sp_C	30.9234, 3.602	0.92	0.80
II	Se_CSCC	10.657, 2.7042	0.85	0.60
	Sp_CSCC	17.1493, 8.9541	0.67	0.50
	Se_C	11.7587, 8.4764	0.59	0.40
	Sp_C	13.0015, 2.0436	0.92	0.70
III	Se_CSCC	1.9989, 1.1763	0.85	0.20
	Sp_CSCC	2.3361, 1.6581	0.67	0.20
	Se_C	2.6352, 2.1363	0.59	0.20
	Sp_C	1.9253, 1.0805	0.92	0.20
IV	Se_CSCC	1, 1		
	Sp_CSCC	1, 1		
	Se_C	1, 1		
	Sp_C	1, 1		

(Greiner et al., 2000). We considered two prevalence scenario, 25% and 75%, and equal and fivefold cost of a FN to FP.

2.5.5. Assessment of convergence

The models were set with 20,000 iterations using the Gibbs sampler and the first 1000 were discarded at the burn-in phase. Three chains with different initial values were used and this was assessed by viewing chain paths and by using the Gelman–Rubin convergence statistic. Autocorrelations were also checked.

2.5.6. Statistical software

The models were run in WinBUGS (Spiegelhalter et al., 1996). The same program was used to check the Gelman–Rubin diagnostic, the autocorrelations and the DIC (DIC Tool dialog box). Beta distributions were determined using the BetaBuster program.

3. Results

One hundred and seventy-five cows were recruited in June 2008 during the herd visits. The proportion of heifers was 10.3% and the median of days in milk was 175. Both herds showed similar patterns regarding parity and days in milk distributions (Table 2).

Fifty-five percent of the cows had CSCC ≥200,000 cells/mL (Table 3) and most of them (76.3%) belonged to Herd 2. Ninety-five percent (95.4%) of the cows studied, had at least one infected quarter either with minor or major pathogens. The prevalence of cows with major pathogen was higher in Herd 2 (Table 3). The 80% of the cows had 1 or 2 quarters infected with major pathogens. Sixty-four percent of the sampled quarters

Table 2
Days in milk and parity distribution for all cows and per herd in a study to detect intra-mammary infections in Argentinian dairy herds.

	All cows (n = 175)	Herd 1 (n = 53)	Herd 2 (n = 122)
Median of days in milk (range)	175 (23–583)	144 (49–330)	183 (23–583)
Heifers	18 (10.3%)	5 (9.4%)	13 (10.8%)

Table 3
Composite SCC ≥200,000 cells/mL and infection with major and minor pathogens distribution for all cows and per herd in a study to detect intra-mammary infections in Argentinian dairy herds.

	All cows (n = 175)	Herd 1 (n = 53)	Herd 2 (n = 122)
CSCC ≥200,000 cells/mL	96 (54.9%)	22 (41.5%)	74 (60.7%)
Infection with major pathogens	104 (59.4%)	13 (24.5%)	91 (74.6%)
Infection with minor pathogens	63 (36.0%)	37 (69.8%)	26 (21.3%)

Table 4
Quarter infection status according to pathogens (n = 684).

Pathogen isolated	Number of quarters (%)
Major pathogens	
Staphylococcus aureus	112 (16.5)
Enterobacteria	34 (5)
Streptococcus agalactiae	14 (2.1)
Environmental streptococci	22 (3.2)
S. aureus – *Streptococcus agalactiae*	4 (0.6)
S. aureus – Environmental streptococci	6 (0.9)
S. aureus – Enterobacteria	3 (0.4)
Pseudomonas spp.	2 (0.3)
Minor pathogens	
Coagulase-negative staphylococci	206 (30.3)
Corynebacterium bovis	29 (4.3)
Coagulase-negative staphylococci – *Corynebacterium bovis*	2 (0.3)
No growth	245 (36.1)

were positive to some pathogen. The proportion of major pathogens was slightly lower than the proportion of minor pathogens (Table 4).

The cross-classified results of milk CSCC and culture by both herds are displayed in Table 5. The threshold where the ROC curve is optimized was 150,000 cells/mL. The estimated Se_CSCC and Sp_CSCC were 0.80 (95% CrI 0.71–0.88) and 0.57 (95% CrI 0.44–0.71), respectively, under the independence model (Table 6). The estimated Se_C and Sp_C for the same model and threshold were 0.83 (95% CrI 0.73–0.93) and 0.89 (95% CrI 0.74–0.98), respectively (Table 6). The estimated median prevalence were 22% (95% CrI 1–38) and 84% (95% CrI 72–93) for Herds 1 and 2, respectively (Table 6). Trade off in Se and Sp was observed for different thresholds between 100,000 and 300,000 cells/mL (Table 6). The Se and Sp posterior distribution for CSCC and culture regarding different thresholds values are displayed in Fig. 1.

Table 5
Cross-classified results of CSCC and culture for herds with low prevalence (Herd 1) and high prevalence (Herd 2) in a study to detect intra-mammary infections in Argentinian dairy herds.

	Culture+	Culture−
Herd 1		
CSCC ≥200,000 cells/mL	6	16
CSCC <200,000 cells/mL	7	24
Herd 2		
CSCC ≥200,000 cells/mL	63	11
CSCC <200,000 cells/mL	28	20

Evidence of conditional dependence between CSCC and culture was not found since all estimates of correlation between tests were low and all probability intervals included zero. In addition, the parameters estimated (Ses, Sps and prevalences) for both models showed no difference regarding 95% CrI (Table 6). The estimated parameters showed little variation considering different prior information (Table 7).

The AUC for CSCC was calculated as 0.72. Regarding costs, different thresholds did not change markedly the MCT, except for the scenario where the prevalence was high and the ratio of cost of FN to FP was equal five (Fig. 2).

4. Discussion

The SCC as diagnostic test gives a quantitative output; therefore, several authors have investigated the appropriate threshold to improve the test's accuracy. The ROC analysis suggested that the most appropriate CSCC threshold would be 150,000 cells/mL, which is lower than 200,000 cells/mL, an operational threshold considered of practical value (Schukken et al., 2003; Lam et al., 2009; Andersen et al., 2010; Dufour and Dohoo, 2013). However, the 150,000 cells/mL criterion is based in a model that account for the classification error of the culture. In contrast, the most applied CSCC thresholds (200,000 cells/mL) to identify IMI is based in the assumption that culture is a

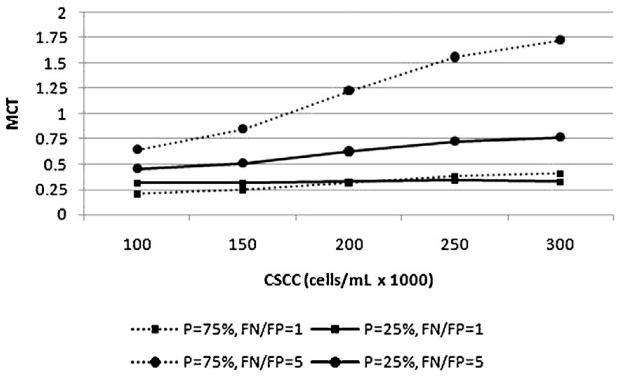

Fig. 2. Misclassification cost term (MCT) for 25% (continues line) and 75% (dotted line) IMI prevalence (P) in Argentinian dairy herds, as a function of CSCC thresholds considering equal (square) and fivefold (circle) cost of a FN to a FP test results.

Table 6
Median and 95% credible intervals (CrI) for Se_CSCC, Sp_CSCC, Se_C, Sp_C and prevalences of intra-mammary infection for major pathogens (P) at five CSCC thresholds in Herds 1 and 2, under the independence and dependence model.

Model	CSCC (cells/mL) thresholds	Median (95% CrI)					
		Se_CSCC	Sp_CSCC	Se_C	Sp_C	P Herd 1	P Herd 2
Independence	100,000	0.85 (0.78–0.92)	0.49 (0.36–0.64)	0.82 (0.72–0.91)	0.91 (0.76–0.99)	0.24 (0.10–0.40)	0.86 (0.74–0.94)
	150,000	0.80 (0.71–0.88)	0.57 (0.44–0.71)	0.83 (0.73–0.93)	0.89 (0.74–0.98)	0.22 (0.07–0.38)	0.84 (0.72–0.93)
	200,000	0.69 (0.59–0.79)	0.66 (0.54–0.78)	0.86 (0.75–0.95)	0.86 (0.71–0.98)	0.19 (0.00–0.35)	0.80 (0.67–0.90)
	250,000	0.59 (0.49–0.70)	0.71 (0.59–0.82)	0.87 (0.75–0.95)	0.87 (0.71–0.98)	0.19 (0.00–0.35)	0.79 (0.67–0.90)
	300,000	0.55 (0.44–0.66)	0.79 (0.68–0.88)	0.86 (0.76–0.95)	0.86 (0.7–0.98)	0.18 (0.00–0.34)	0.79 (0.67–0.90)
Dependence	100,000	0.84 (0.76–0.91)	0.48 (0.34–0.64)	0.79 (0.69–0.88)	0.88 (0.75–0.96)	0.24 (0.10–0.40)	0.87 (0.75–0.95)
	150,000	0.78 (0.69–0.87)	0.56 (0.42–0.71)	0.8 (0.7–0.89)	0.87 (0.74–0.96)	0.22 (0.09–0.38)	0.86 (0.74–0.95)
	200,000	0.66 (0.56–0.77)	0.65 (0.51–0.77)	0.8 (0.7–0.89)	0.85 (0.72–0.95)	0.20 (0.08–0.35)	0.85 (0.72–0.95)
	250,000	0.56 (0.46–0.67)	0.69 (0.55–0.81)	0.8 (0.7–0.89)	0.85 (0.72–0.95)	0.20 (0.08–0.36)	0.85 (0.72–0.95)
	300,000	0.52 (0.41–0.63)	0.78 (0.65–0.88)	0.8 (0.7–0.89)	0.85 (0.71–0.95)	0.19 (0.07–0.36)	0.85 (0.71–0.95)

perfect test, which disregards the chances of false negative results to CSCC lower than 200,000 cells/mL.

The 200,000 cells/mL threshold has been evaluated to identify cows with IMI associated with the major pathogens (McDermott et al., 1982; Dohoo and Leslie, 1991). The estimated Se_CSCC was lower than that reported by McDermott et al. (1982) and Dohoo and Leslie (1991), who found a Se and Sp of 0.89 and 0.83, respectively. The estimated Sp_CSCC value was among those reported by McDermott et al. (1982) and Dohoo and Leslie (1991). In all cases, culture was considered the reference test; however, differences in the figures estimated in this research may be related to the model used, which regard to culture as imperfect test. The culture accuracy for all major pathogens was investigated previously by Sanford et al. (2006), who reported a lower Se (0.56) and a slightly higher Sp (0.93), using CMT instead of CSCC as a second test. Such differences may be due to the fact that SCC distribution within the CMT lower categories would be overlapped (Ruegg and Reinemann, 2002). The Se_C and Sp_C estimated could also differ because Sanford et al. (2006) worked with cow at dry off as reference population, an aspect that has been regarded as critical in validation studies (Greiner and Gardner, 2000). The Se_C and Sp_C were similar and overlapped the posterior distributions across all thresholds evaluated in the CSCC range from 100,000 to 300,000 cell/mL (Fig. 1). This may have been related to the fact that the validation involved only major pathogen, which can induce SCC over 350,000 cells/mL (Djabri et al., 2002).

The subclinical mastitis has been classified as acute, subacute or chronic (National Mastitis Council, 2003). This report relates to cross-sectional data, therefore to infer the proportion of each type of mastitis is not possible. However, most of the major pathogens isolated were contagious (e.g., *S. aureus*), and such IMIs are often characterized by their long duration (Sears et al., 1990; De Haas et al., 2004); this could be a plausible explanation for the coexistence of high SCC and pathogens in the same cow.

Although we considered the classification error and the correlation of both tests, the proportion of false positive and false negative cows was still considerable. Cows with CSCC <150,000 cells/mL and isolation of major pathogens in at least one quarter (false negative cows) could be attributed to the dilution effect produced by milk of non-infected quarters (Dohoo and Meek, 1982). This may be important because in this study most of the cows showed only one or two quarters infected. These cows showed a numerical trend toward lower of the CSCC median in comparison to cows with three or four infected quarters (data not shown).

On the other hand, a false positive result was represented by a cow with CSCC ≥150,000 cell/mL and no quarter infected with major pathogen. More than 90% of these false positive cows were positive to minor pathogens, a fact that can partially explain why SCC is above 150,000 cells/mL in the composite sample. Quarters with CNS IMI had an SCC almost twice as high as culture-negative quarters (Sampimon et al., 2009). Another alternative explanation for false positive results would be that the SCC of cows that have IMI for contagious pathogens take long to stabilize at

Table 7
Median and 95% credible intervals (CrI) for Se_CSCC, Sp_CSCC, Se_C and Sp_C at five CSCC thresholds using different sets of prior information, under the independence model.

CSCC thresholds	Set prior	Median (CrI 95%)			
		Se CSCC	Sp CSCC	Se Culture	Sp Culture
100,000 cells/mL	I	0.85 (0.78–0.91)	0.64 (0.56–0.71)	0.70 (0.63–0.76)	0.91 (0.82–0.97)
	II	0.85 (0.78–0.91)	0.55 (0.42–0.68)	0.78 (0.69–0.86)	0.91 (0.78–0.98)
	III	0.85 (0.77–0.92)	0.45 (0.32–0.61)	0.84 (0.73–0.95)	0.90 (0.73–0.99)
	IV	0.85 (0.77–0.92)	0.43 (0.30–0.59)	0.87 (0.75–0.99)	0.91 (0.72–1.00)
150,000 cells/mL	I	0.80 (0.72–0.87)	0.65 (0.58–0.72)	0.71 (0.64–0.77)	0.90 (0.79–0.97)
	II	0.80 (0.71–0.87)	0.60 (0.48–0.72)	0.79 (0.70–0.88)	0.89 (0.76–0.98)
	III	0.79 (0.70–0.88)	0.54 (0.40–0.69)	0.86 (0.75–0.96)	0.88 (0.70–0.99)
	IV	0.80 (0.71–0.88)	0.52 (0.39–0.67)	0.89 (0.77–0.99)	0.89 (0.70–0.99)
200,000 cells/mL	I	0.69 (0.61–0.78)	0.67 (0.60–0.74)	0.72 (0.65–0.78)	0.88 (0.77–0.96)
	II	0.69 (0.59–0.79)	0.67 (0.56–0.78)	0.81 (0.72–0.9)	0.87 (0.73–0.97)
	III	0.69 (0.58–0.80)	0.65 (0.53–0.78)	0.89 (0.78–0.98)	0.85 (0.68–0.98)
	IV	0.69 (0.58–0.80)	0.64 (0.52–0.77)	0.93 (0.80–1.00)	0.85 (0.68–0.99)
250,000 cells/mL	I	0.61 (0.52–0.70)	0.68 (0.61–0.75)	0.72 (0.64–0.78)	0.88 (0.76–0.96)
	II	0.59 (0.50–0.70)	0.71 (0.59–0.80)	0.82 (0.72–0.90)	0.87 (0.72–0.97)
	III	0.59 (0.48–0.70)	0.71 (0.59–0.82)	0.90 (0.78–0.98)	0.86 (0.68–0.99)
	IV	0.59 (0.48–0.70)	0.70 (0.58–0.81)	0.94 (0.80–1.00)	0.86 (0.68–0.99)
300,000 cells/mL	I	0.56 (0.47–0.65)	0.71 (0.64–0.77)	0.72 (0.64–0.79)	0.87 (0.75–0.96)
	II	0.55 (0.45–0.65)	0.77 (0.66–0.86)	0.82 (0.72–0.90)	0.86 (0.71–0.97)
	III	0.54 (0.43–0.65)	0.80 (0.69–0.90)	0.89 (0.77–0.98)	0.85 (0.68–0.98)
	IV	0.54 (0.43–0.66)	0.80 (0.68–0.89)	0.93 (0.80–1.00)	0.85 (0.68–0.99)

the lowest level (Pyörälä and Pyörälä, 1997; De Haas et al., 2002, 2004).

The latent class model assumes that each test evaluated provides information about the same latent condition (Enøe et al., 2000). In this case, CSCC and culture are combined in the latent class model, the definition of the latent disease being based on information provided by both tests. As a result of that, the accuracy to detect IMI will be related to the performance of each individual test and their combination. The existence of conditional dependence between both test results influences their ability to detect infected (or non-infected) animals (Gardner et al., 2000). Since no correlation between tests was detected, both can be used separately or sequentially with no major concerns about bias in the accuracy estimates (Gardner et al., 2000).

The AUC represents a summary statistic of the overall diagnostic performance of the test (Greiner et al., 2000). The AUC estimated was of 0.72, which was a sign of moderate accuracy (Swets, 1988). The relevance of knowing the CSCC accuracy to detect IMI is that it would provide information about the subclinical mastitis prevalence (Schukken et al., 2003). In addition, this information is beneficial to use CSCC as tool for mastitis management in the herds. The choice of the CSCC threshold value is important because the CSCC level affects the proportion of correctly and incorrectly classified samples (Greiner and Gardner, 2000; Ruegg and Reinemann, 2002). The thresholds selection is a decision that should take into account the herd prevalence and the cost of false negative and false positive results (Greiner and Gardner, 2000). Considering the CSCC test for udder health management purposes, in herds where udder infection is under control (low prevalence), the NPV of CSCC would be high. Under this scenario, a CSCC at low threshold (e.g., 150,000 cells/mL) could be considered as a cost-effective prescreening sampling tool to determine the pathogen profile of a herd, as suggested by Reyher et al. (2011). Composite SCC can be complemented with milk culture as a second test, to reduce false positives (Koop et al., 2011). In addition to this, CSCC at low threshold could help to identify non-infected cows in the context of the design of a selective dry cow therapy (Østeråas et al., 1999; Halasa et al., 2010). Under this scenario, the cost ratios of FN to FP evaluated did not restrict the selection of a CSCC threshold. In contrast, when prevalence is high, the PPV of CSCC is also high; however, lower threshold would still the choice, especially when the cost ratio of FN to FP was five. This is because both Se and NPV should be increased to reduce the cost of misclassification. Within this framework, different decisions could be taken to manage early dry off, culling or milking order.

This was a cross-sectional study; however, the accuracy of a test could be eventually modified by sampling individuals several times (Dohoo et al., 2003). The use of consecutive CSCC records makes the evaluation of the cow udder health status more reliable (Pyörälä, 2003) and this should be evaluated in futures research. In the same way, other criteria for diagnosing of IMI such as the number of colonies of the pathogens of interest, the use of duplicate or triplicate milk sample and, other issues previously evaluated (Dohoo et al., 2011), should be assessed using latent class model in order to expand the understanding the CSCC accuracy.

Our analysis depends on three key model assumptions. First, the two test outcomes for a given individual are conditionally independent of disease status. Second, the Se and Sp of the two tests remain unchanged across populations. Finally, the two populations have different prevalences (Christensen et al., 2010).

The results of conditional dependence models showed that both tests are conditionally independent. In our study, this is reasonable because the two tests measure different biological processes (Gardner et al., 2000; Georgiadis et al., 2003). The CSCC measures inflammatory response which implies polymorphonuclear neutrophil migration to site of infection (Sordillo, 2005), whereas bacteriological

culture relies on the isolation of the pathogen involved in IMI. Furthermore, evidence of conditional dependence between culture and tests that measures the inflammatory response in udder has not been demonstrated previously in cows (Sanford et al., 2006) and goats (Koop et al., 2011).

The constant accuracy among herds was evaluated performing a separate analysis of tests to evaluate the variation of the parameters (Johnson et al., 2009) and the sensitivity analysis. In addition, biological factors such as parity and DIM, affect the distribution of the test value (Dohoo et al., 1981; Schepers et al., 1997; Laevens et al., 1997) and consequently, also the diagnostic Se and Sp (Greiner and Gardner, 2000; Dohoo et al., 2003). The distributions of these variables were evaluated among herds studied, and no important differences were observed. Thus, we assume that there would be no great influence on the estimated values of Se and Sp.

To accomplish the last assumption, the prevalence status was assessed using bulk milk SCC, since this is a good indicator of number and duration of infections present plus rate of new infections. A low bulk milk SCC consistently correlates with low level mammary gland inflammation (Eberhart et al., 1982; Brolund, 1985; Reneau, 2001).

The accuracy estimates reported here are only valid for major pathogen quarter infections. In addition to that, the lack of cows with negative culture quarters did not allow the estimation of the Se_CSCC and Sp_CSCC for all pathogens.

5. Conclusions

To the best of our knowledge, this is the first report of the CSCC accuracy to detect IMI for major pathogens considering the culture misclassification. No correlation between the CSCC and culture was detected, so that the estimates from the independence model could be properly applied. Based on ROC analysis the thresholds that maximize the Se and Sp CSCC would be 150,000 cells/mL. This criterion remained stable beyond the different FN:FP cost ratio scenarios evaluated.

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