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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