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Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle

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ABSTRACT

Screening tests for mastitis can play an important role in proactive mastitis control programs. The primary objective of this study was to compare the sensitivity and specificity of milk electrical conductivity (EC) to the California mastitis test (CMT) in commercial dairy cattle in South Africa using Bayesian methods without a perfect reference test. A total of 1848 quarter milk specimens were collected from 173 cows sampled during six sequential farm visits. Of these samples, 25.8% yielded pathogenic bacterial isolates. The most frequently isolated species were coagulase negative Staphylococci ($n = 346$), *Streptococcus agalactiae* ($n = 54$), and *Staphylococcus aureus* ($n = 42$). The overall cow-level prevalence of mastitis was 54% based on the Bayesian latent class (BLC) analysis.

The CMT was more accurate than EC for classification of cows having somatic cell counts >200,000/mL and for isolation of a bacterial pathogen. BLC analysis also suggested an overall benefit of CMT over EC but the statistical evidence was not strong ($P = 0.257$). The Bayesian model estimated the sensitivity and specificity of EC (measured via resistance) at a cut-point of >25 mΩ/cm to be 89.9% and 86.8%, respectively. The CMT had a sensitivity and specificity of 94.5% and 77.7%, respectively, when evaluated at the weak positive cut-point. EC was useful for identifying milk specimens harbouring pathogens but was not able to differentiate among evaluated bacterial isolates. Screening tests can be used to improve udder health as part of a proactive management plan.

Introduction

Mastitis is one of the most costly diseases of dairy cattle and has major importance for the commercial dairy sector in South Africa. In 2009, there were 3551 commercial milk producers in the country (MPO, 2009) with a median herd size of 145 cows (MPO, 2010). The recorded average daily milk production per cow was 17.8 kg and in 34% of herds, cows averaged between 15.6 and 20.6 kg and in 19.4% of herds >20.6 kg (MPO, 2010). Twenty-four percent of South African dairy cattle (129,511 cows in 656 herds) were monitored in 2008 by the National Milk Recording Scheme and the average yield per lactation for recorded cows was 7271 kg which was approximately 50% higher than the national herd average (du Toit, 2009). Holsteins total 47.5% of the monitored dairy cattle and another 45.2% were Jersey with the remainder predominantly Ayrshire (5.5%) (du Toit, 2009).

In South Africa, *Streptococcus agalactiae* and *Enterococcus canis* have been associated with clinical mastitis problems within herds (Petzer et al., 2009). Coagulase-negative staphylococci were the most frequently isolated bacteria in milk samples from both lactating and dry cows during 2000–2007, followed by *Staphylococcus aureus* and *Streptococcus agalactiae* (Petzer et al., 2009). *Staphylococcus aureus* can be considered the most important mastitis pathogen in South Africa because of its economic impact (Wilson et al., 1997) and potential for chronic infections and treatment failures.

A number of diagnostic options exist for the identification of mastitis but they have differences with respect to accuracy (sensitivity and specificity) and cost (Emanuelson et al., 1987; Pyorala, 2003; Viguier et al., 2009). A difficulty in the estimation of diagnostic sensitivity and specificity is the lack of a gold standard for the classification of cattle as having mastitis (Dohoo et al., 2011). The enumeration of somatic cells is a common method for identification of gland inflammation and is frequently approximated on an ordinal scale using the California mastitis test (CMT) (Schalm and Noorlander, 1957). Electrical conductivity (EC), or resistance as the inverse, has also been employed to detect mastitis (Fernando et al., 1982; Nielen et al., 1992; Norberg et al., 2004) and hand-held meters have been promoted as a screening tool in South Africa. Milk EC is mainly determined by type and concentration of ions, interactive influence of the ions, and components contributing to milk viscosity including protein, fat, and lactose (Henningsson et al., 2005).

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The objective of the present study was to compare the sensitivity and specificity of milk EC to the CMT for the identification of mastitis in commercial dairy cattle in South Africa using Bayesian methods without a perfect reference test. Secondary objectives included the evaluation of diagnostic methods based on a microbiological classification of mastitis and to determine whether resistance values varied by microbiological results. We hypothesized that the sensitivity of CMT would be greater than EC based on biological definitions of mastitis but not when the analysis was performed in the absence of an assumed gold standard.

Materials and methods

Herd sampling

All farms were visited during 2008 upon request by the producer and were within a 100 km radius of Onderstepoort in Gauteng province. Herds were visited a maximum of six times to monitor effectiveness of interventions (data not presented). Quarter milk samples were collected after clinical examination using a strip cup. Samples were maintained on ice and transported to the Milk Laboratory, Production Animal Studies, Onderstepoort for analysis. Diagnostic procedures were performed as a service to producers and statistical analyses were performed after extraction of recorded data.

Laboratory testing

Milk electrical conductivity (EC)

Approximately 5 mL of milk were ejected into the cup of a hand-held conductivity meter (Mast-O-Test, Durotec) after collection of specimens for bacterial culture. Milk electrical resistance (MER) readings were obtained independently from each quarter.

California mastitis test (CMT)

The CMT was performed immediately after recording MER. Results were recorded as 0, negative; 1, weak positive; 2, distinct positive; and 3, strong positive in accordance with the manufacturer's guidelines (California mastitis test kit, ImmuCell).

Somatic cell counts (SCCs)

Quarter-level somatic cell counting was performed using a Fossomatic 90 cell counter (FOSS Analytical).

Organism isolation

Quarter milk samples were plated onto Columbia Agar base supplemented with 5% defibrinated bovine blood and incubated aerobically for 24–48 h at 37 °C. Isolated bacteria were identified based on colony morphology, haemolysis, catalase, potassium hydroxide test and Gram staining. Additional tests included the latex agglutination Strepkit (Quantum Biotechnologies), Staphylase Test (Quantum Biotechnologies) and the API 20E kit (Omnimed).

Statistical analysis

Descriptive statistics for SCC, milk EC, and the CMT were calculated as the median and interquartile range (IQR; 25th to 75th percentile). Diagnostic sensitivity (Se) and specificity (Sp) of the milk EC and CMT were estimated relative to two classifications of mastitis, namely, (1) a quarter-level SCC > 200,000/mL (Schukken et al., 2003), and (2) the successful isolation of a pathogenic bacteria. Only cows sampled during the first herd visit ($n = 173$) were used to evaluate Se and Sp: Se was estimated at the quarter-level as the proportion of positive test results within those quarters that were classified as having mastitis; Sp was similarly estimated within quarters that were mastitis negative. The design effect (Ukoumunne, 2002) was estimated to adjust for the clustering of quarters within cows and available software (Epi Info version 6.04d for Windows, Centers for Disease Control and Prevention) was used to calculate 95% confidence intervals (CIs). A Monte Carlo simulation method was employed to estimate and compare area under the receiver-operating characteristic curve (AUC).

Diagnostic Se and Sp of milk EC (via measured MER) and CMT were estimated within a Bayesian framework using a two-test, four population model assuming conditional independence. Each quarter was considered a separate population to eliminate the problem of interdependence of mastitis among quarters (Barkema et al., 1997). Tests were evaluated as ordinal results rather than dichotomization as positive or negative. Milk electrical resistance was categorized into three ordinal levels (<25 mΩ/cm, 25–30 mΩ/cm, and >30 mΩ/cm) based on manufacturer's suggestion of a green light being a healthy udder (>30 mΩ/cm), yellow light indicating a mastitis suspect (25–30 mΩ/cm), and red light a mastitis positive (<25 mΩ/cm). The CMT was evaluated using the typical scores of 0–3. Only cows sampled during the first herd visit and with complete test information were included in this analysis ($n = 168$). Non-informative prior probability distributions (beta 1, 1) were employed for components of Se and Sp and a mildly informative prior was used for prevalence (beta 4.7, 10.4). The diffuse prior for prevalence was determined independent of a single test result and based on the quarter-level mastitis prevalence (31%; 212/683) using the number of cows with SCC > 400,000/mL and isolation of bacterial pathogen as an approximate gold standard (Petzer et al., 2009). Receiver-operating characteristic (ROC) curves were plotted for evaluated cut-offs by connecting the points of the 1 − Sp (*X*-axis) by Se (*Y*-axis). Area under the estimated ROC curve was calculated by the trapezoid approximation method (Munem and Foulis, 1984). Similar Bayesian latent class (BLC) analyses have been previously described (Fosgate et al., 2007, 2010).

Markov chain Monte Carlo (MCMC) techniques were employed using available statistical software (WinBUGS Version 1.4, MRC Biostatistics Unit). Autocorrelation among iterate values was assessed and only every fifth value was retained. Convergence was assessed by evaluating plots of model parameter iterates and by calculating the Gelman–Rubin statistic. The first 200,000 iterations were discarded as the burn-in and inferences were made based on the subsequent 40,000. Median values were used as point estimates and 95% probability intervals (PIs) were calculated as the 2.5th to 97.5th percentiles of the posterior distribution.

A random effects variance component analysis was performed to determine the proportion of variability in EC measurements due to bacterial isolate, cow, and quarter-level factors incorporating results from all herd visits. Mixed effects linear regression was used to determine if EC values varied by bacterial isolate while adjusting for cow as a random effect and quarter as a fixed effect with Bonferroni adjustment for multiple post hoc pairwise comparisons. For this component of the analysis isolates were grouped as none, coagulase negative Staphylococci, *Streptococcus agalactiae*, *Staphylococcus aureus*, and other organisms based on the empirical distribution of counts in the sample. Contaminated ($n = 3$) and mixed growth ($n = 3$) cultures were excluded. Statistical modelling was performed in commercially available software (SPSS version 17.0, SPSS).

Results

A total of 1858 quarter milk specimens with complete data were collected from 173 cows sampled during six sequential farm visits. Four hundred and seventy-seven specimens (25.8%) yielded pathogenic bacteria and test results varied between isolates (Table 1). One hundred and sixty-eight cows sampled during the first visit had complete test information for all four quarters. During the first visit, 81% of sampled cows had at least one quarter with SCC > 200,000/mL and 59% of cows yielded pathogenic bacteria from at least one quarter (Table 2). The overall cow-level prevalence of mastitis during the first visit was estimated to be 54% based on the BLC analysis and the prevalence varied by quarter with the right side of the udder more likely to be affected.

Se and Sp of milk EC and CMT were estimated over the ordinal categories and descriptively varied based on the evaluated definitions of mastitis (Table 3). Sensitivities were noticeably higher when estimated via BLC analysis. Latent class analytic results also suggested that the tests overall were more accurate when contrasted with the other definitions of mastitis (Table 4). The CMT was more accurate than EC for classification of quarters with SCC > 200,000/mL and isolation of a pathogen. The overall accuracy (Se and Sp over all cut-offs) of CMT was descriptively different than EC based on the BLC analysis but not statistically better (AUC 0.931 vs. 0.904; $P = 0.257$). Receiver-operating characteristic curves plotted at the evaluated cut-points did not suggest large differences in overall accuracy for EC and CMT (Fig. 1).

Variance components analysis of all 1858 quarter-milk specimens estimated the amount of variability in EC due to cow factors, quarter factors, and bacterial isolation results as 20.4%, 0.1%, and 2.5%, respectively. Seventy-seven percent of the variability in EC was unexplained by these variables. Bacterial species were categorized as none ($n = 1371$), coagulase negative Staphylococci ($n = 346$), *Streptococcus agalactiae* ($n = 54$), *Staphylococcus aureus* ($n = 42$), and other ($n = 39$). Mixed effects linear regression of EC estimated significant effects for cow ($P < 0.001$) and bacterial category ($P < 0.001$) but not for quarter ($P = 0.222$). Post hoc pairwise comparisons demonstrated lower EC for coagulase negative Staphylococci and *Streptococcus agalactiae* compared to no bacterial iso-