ABSTRACT

The main objective of this study was to investigate the diagnostic test performance of somatic cell count (SCC), lactate dehydrogenase (LDH), and \( N\)-acetyl-\( \beta\)-d-glucosaminidase (NAGase), analyzed in composite test milking samples, for detecting dairy cows with or without intramammary infection (IMI). A second objective was to investigate whether an adjustment of these udder health indicators according to their associations with different influential factors (i.e., parity, days in milk, and season) improved their test performance. Moreover, we wanted to investigate whether test performance of SCC improved if SCC results from previous adjacent test milkings were included in the model. Such test milking data were not available for LDH or NAGase. In this cross-sectional study, quarter milk samples for bacteriological examination were taken from almost 1,000 cows from 25 dairy herds during 3 consecutive days: the day before test milking, the day of test milking, and the day after test milking. From each cow, a composite test milking sample was analyzed for milk composition, SCC, LDH, and NAGase. Among the cows sampled, 485 were IMI negative and 256 were IMI positive in one or more udder quarters according to the definitions used. The remaining cows had inconclusive IMI status. To assess the test performance of SCC, LDH, and NAGase to identify IMI-negative and IMI-positive cows, univariable generalized estimating equation models were used with the udder health indicator of interest as outcome and IMI status as explanatory variable. From these models, receiver-operator characteristic curves were created and the area under cure (AUC) was calculated. From each model, a cut-off was chosen for calculations of the sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (ACC) for each udder health indicator. The AUC was similar for the adjusted SCC (0.84), nonadjusted SCC (0.83) and geometric mean SCC (0.80–0.81), but much lower for LDH (0.66) and NAGase (0.62). The highest Se, Sp, PPV, NPV, and ACC were obtained using SCC. Adjustment of the udder health indicators for influential factors (e.g., parity) did not improve the test performance markedly, whereas adding information about SCC from previous adjacent test milkings improved the test performance of SCC slightly. In conclusion, of the udder health indicators investigated, SCC had the best overall ability to correctly identify IMI-negative and IMI-positive dairy cows.

Key words: sensitivity, specificity, udder health indicator, intramammary infection

INTRODUCTION

One of the most common diseases of dairy cows is mastitis. In most cases, mastitis is caused by bacteria, and detection of cows with bacterial IMI is an important part of preventive udder health management to reduce the spread of udder pathogens in the herd. Today, the best available method to identify cows with IMI is to collect quarter milk samples for bacterial culture or testing by PCR. However, both methods are expensive when the aim is to sample all udder quarters of all cows in a herd. An indirect way to identify cows with IMI is to measure inflammatory indicators (i.e., udder health indicators) in milk such as SCC, lactate dehydrogenase (LDH), or \( N\)-acetyl-\( \beta\)-d-glucosaminidase (NAGase; Sandholm et al., 1995). New, more efficient, and economically viable methods to analyze the activity of LDH and NAGase have been developed (Larsen, 2005; Larsen et al., 2010), and LDH is included in the milk analysis tool Herd Navigator (DeLaval, Tumba, Sweden). However, the most common way to evaluate inflammation in the udder is to analyze SCC in cow composite milk samples taken at monthly test milkings. Cows with suspected IMI, indicated by a high composite milk SCC (CMSCC), can then be sampled at the quarter level for culturing or PCR testing to identify IMI-positive quarters.
When using udder health indicators to find cows with IMI, it is important to know the test performance of the indicators. Several studies have investigated the sensitivity (Se) and specificity (Sp) of CMSCC in detecting cows with and without IMI using bacteriology as the gold standard (Dohoo and Leslie, 1991; Reksen et al., 2008; Reyher and Dohoo, 2011). In those studies, Se and Sp varied from 25 to 77% and from 62 to 100%, respectively. Two recent studies (Mahmmod et al., 2013; Vissio et al., 2014) that included bacteriological culture and PCR testing results used latent class analysis to estimate the Se and Sp of SCC. They found that the estimated Se and Sp in SCC in classifying cows with or without IMI varied between 61 and 80% and between 57 and 65%, respectively. These studies used somewhat different definitions of IMI or only investigated the Se and Sp for specific bacteria (e.g., *Staphylococcus aureus*). However, the results from these studies indicate that many cows would be classified as either false negative or false positive with respect to IMI by just assessing CMSCC. Adjusting the CMSCC for influential factors, such as parity, breed, and season, as well as adding information about the CMSCC at previous adjacent test milkings might improve the test performance. However, to our knowledge, only a few studies have investigated this (Reksen et al., 2008; Reyher and Dohoo, 2011), and more information is needed to assess whether test performance of SCC would improve by adjustments according to influential factors or by adding information from previous adjacent test milkings.

Other udder health indicators, adjusted or not adjusted for influential factors, might have a better test performance than SCC. Both LDH and NAGase have been shown to be associated with mastitis and IMI (Bogin and Ziv, 1973; Nielsen et al., 2005; Chagunda et al., 2006; Babaei et al., 2007). Moreover, Mattila et al. (1986a) found that NAGase was better than SCC in identifying IMI-positive quarters. However, to our knowledge, the test performance of LDH and NAGase for classifying cows with or without IMI has not been published. Babaei et al. (2007) investigated how the Se and Sp changed for different cut-offs of LDH in identifying udder quarters with subclinical mastitis (defined by California Mastitis Test) but not in identifying cows with IMI. Moreover, we are unaware of any publications showing that the test performance of LDH and NAGase would improve after adjustment for influential factors.

The main aim of this project was to investigate the test performance of SCC, LDH, and NAGase in cow composite milk from one test milking in detecting IMI-negative and IMI-positive cows. The second aim was to determine if a preadjustment of these udder health indicators for influential factors would improve the diagnostic ability. Moreover, as information on CMSCC from previous test milkings was available, we also wanted to investigate whether the diagnostic ability of SCC would improve if such information were included.

**MATERIALS AND METHODS**

The material has previously been described in detail (Nyman et al., 2014). In brief, 25 dairy herds were recruited for this repeated cross-sectional study. The herds had an annual herd size of 60 to 200 dairy cows, had 30% or more of each of the main Swedish dairy breeds (i.e., Swedish Red and Swedish Holstein), and had an estimated bulk milk SCC of 150,000 to 300,000 cells/mL. Each herd was visited twice during the housing season from October 2009 to April 2010. At these visits, a technician attended and sampled cows at one milking occasion (morning or evening) on the day before test milking, on the day of test milking, and on the day after the test milking. The intention was to sample 20 cows at each visit, resulting in 40 different cows being sampled in each herd. Before the visits, cows to be sampled in each herd were randomly selected among available cows, ensuring equal distribution within breed, parity, and DIM.

**Sampling**

Milk samples for bacteriological examination were taken aseptically just before machine milking at each attended milking. The technicians sampled each udder quarter of each cow at all 3 milking occasions using test tubes. Mastistrip cassettes (SVA, Uppsala, Sweden; Nilsson et al., 1990; Nilsson, 1994; Artursson et al., 2010) were used as transport medium because, at the time of the project, they were commonly used in Sweden. To ensure equal amounts of milk on the 4 filter discs of the Mastistrip cassette, the filter discs were dipped in the milk in the test tubes after sampling. Thereafter, the Mastistrip cassettes were immediately sent to the National Veterinary Institute (Uppsala, Sweden) for analyses.

Cow composite milk samples for analysis of SCC were taken on the day of test milking and handled according to normal routine at test milking. However, in addition to the normal routine, an additional aliquot of the test milk sample was poured into a test tube for analysis of LDH and NAGase.

**Milk Analyses of SCC, LDH, and NAGase**

Cow composite test milk samples were analyzed for SCC (cells/mL) according to normal routines at Steins Laboratory (Jönköping, Sweden) using a Fos-
somatic 5000 cell counter (Foss, Hillerød, Denmark). The samples for analysis of LDH and NAGase were frozen at −20°C, and sent to the Faculty of Agricultural Science, Aarhus University (Foulum, Denmark). Enzyme activities were determined by kinetic fluorometric measurements. Lactate dehydrogenase activity (U/L) was analyzed according to Larsen (2005), and NAGase activity (U/L) was analyzed according to Larsen et al. (2010).

**Bacterial Culture.** Bacteriological analysis of quarter milk samples was performed according to accredited routines at the National Veterinary Institute (Uppsala, Sweden). The filter discs of the Mastistrip were removed on arrival to the laboratory, and each disc was placed in 0.5 mL of RPMI-1640 cell culture medium (SVA) in a test tube. The test tubes were then gently shaken for 10 min at room temperature and thereafter incubated for 18 h at 37°C. After incubation, 50 μL of the milk/medium mixture (corresponding to 10 μL of milk) was cultured on blood (5%) agar plates with esculin, which were incubated at 37°C for 16 to 24 h and re-evaluated at 48 h. A milk sample was classified as IMI negative if <1 cfu of *Staphylococcus aureus* or *Streptococcus agalactiae* was isolated. For other bacteria, a milk sample was classified as IMI negative if <3 cfu were present. Samples were classified as contaminated when 3 or more bacterial types were isolated from a milk sample and if growth of a major udder pathogen (Radostits et al., 2007) was not identified. The only minor udder pathogen (Radostits et al., 2007) reported by the laboratory was CNS.

**Defining IMI Status.** Cows with 12 IMI-negative udder quarter samples and cows with 11 IMI-negative udder quarter samples, and 1 sample with sparse (<1,000 cfu/mL) growth of mixed flora were considered IMI negative. Cows with findings of the same pathogen in the same quarter at 2 or 3 out of 3 sampling occasions or a finding of moderate to rich growth (≥1,000 cfu/mL) of a pathogen in one udder quarter on ≥1 sampling occasion were considered IMI positive. Cows that did not fit these definitions were defined as having inconclusive findings.

**Cow Data.** Individual data on SCC, milk yield (kg of milk), percentage of milk fat and milk protein, and milk urea concentration (mmol/L) from the test milking at sampling and from the 2 previous, most adjacent, test milkings, as well as data on breed, parity, and day of calving for each cow were obtained from the Swedish official milk recording scheme.

**Statistical Analyses**

To assess the test performance of SCC, LDH, and NAGase to identify IMI-negative and IMI-positive cows, univariable generalized estimating equations (GEE) models were used, with a logistic link function and an exchangeable correlation structure to adjust for the within-herd correlation, with the udder health indicator of interest as outcome and IMI status as explanatory variable. All udder health indicators were dichotomized, using a wide range of different cut-offs, and analyzed in one GEE model per cut-off. The sensitivity and 1 − specificity of each cut-off were derived (Coughlin et al., 1992) and used to create receiver-operator characteristic (ROC) curves, one for each udder health indicator. The area under the curve (AUC) for each curve was then calculated using the trapezoid rule.

**Adjustments for Influential Factors.** Adjusted values of SCC, LDH, and NAGase were derived using associations found between the udder health indicators and different factors in a previous study (Nyman et al., 2014). The equations for the adjustments are presented in the Appendix. The adjusted udder health indicators were analyzed in the same way as the unadjusted indicators.

**Inclusion of Repeated Measurements of SCC.** Composite milk SCC, but not LDH or NAGase, was available from previous test milkings. These records were used to evaluate if inclusion of SCC from previous adjacent test milkings improved the test performance of SCC. The SCC from the test milking at bacterial sampling were combined with the SCC from the most previous adjacent test milking as a geometric mean to create a new SCC outcome: GM2SCC. A second geometric mean (GM3SCC) was made by combining the SCC from the test milking at bacterial sampling and the SCC from the 2 most previous adjacent test milkings. Both of these new outcomes were analyzed in the same way as the unadjusted and adjusted indicators. Some cows did not have records from previous test milkings so only cows with records from the 2 previous adjacent test milkings (n = 525) were used in the GM2SCC and GM3SCC models. To get a more correct comparison between GM2SCC, GM3SCC, and the SCC from the test milking at bacteriological sampling, a new outcome was made from the SCC at the test milking at bacteriological sampling only including the same 525 observations as in the GM2SCC and GM3SCC outcomes. The new SCC outcome (SCC525) was analyzed in the same way as the other outcomes.

**Test Performance.** For each udder health indicator, Se, Sp, positive predictive value (PPV; number of IMI-positive cows that test positive/total number of test-positive cows), negative predicted value (NPV; number of IMI-negative cows that test negative/total number of test-negative cows), and accuracy (ACC; total number of IMI-positive cows that test positive and IMI-negative cows that test negative/total num-
ber of cows) were calculated for both nonadjusted and adjusted values and for GM2SCC and GM3SCC. For calculations of test performance, the cut-off with the highest point estimate of \((Se + Sp)/2\) was chosen from each of the models of the nonadjusted and adjusted udder health indicators, as well as from the models of GM2SCC and GM3SCC. The Se and Sp for these cut-offs were also used to calculated PPV, NPV, and ACC for different prevalences (0, 10, 20, 30, 40, or 50% prevalence) for lnSCC, adjusted SCC, and GM2SCC and GM3SCC.

All statistical analyses were performed using Stata Software (release 13.1, 2010; StataCorp., College Station, TX).

RESULTS

Bacteriological Findings

In total, 11,867 milk samples from 976 cows were analyzed, and details of the bacteriological findings are presented in Nyman et al. (2014). Of the 976 cows, 934 had complete data on SCC, LDH, and NAGase from the test milking at the bacterial sampling and were <366 DIM. Of these 934 cows, 489 (52%) were classified as IMI negative, 257 (27%) were classified as IMI positive, and 188 (20%) as having inconclusive findings.

Test Performance

The ROC curves for SCC, LDH, and NAGase are presented in Figure 1. The AUC of these curves was 0.83 [SE (area) = 0.22], 0.66 [SE (area) = 0.11], and 0.62 [SE (area) = 0.07] for SCC, LDH, and NAGase, respectively. The ROC curves for the adjusted values of SCC, LDH, and NAGase are presented in Figure 2. The AUC of these curves was 0.84 [SE (area) = 0.23] for the adjusted SCC, 0.66 [SE (area) = 0.10] for the adjusted LDH, and 0.60 [SE (area) = 0.05] for the adjusted NAGase. The ROC curves for the models where the geometric mean SCC were used, including a ROC curve of only SCC, are presented in Figure 3. The AUC of these curves was 0.80 [SE (area) = 0.22] for GM2SCC, and 0.81 [SE (area) = 0.23] for GM3SCC. The AUC of the SCC525 was 0.84 [SE (area) = 0.23].

The chosen cut-offs and the Se, Sp, PPV, NPV, and ACC for each udder health indicator, nonadjusted and adjusted, as well as for GM2SCC and GM3SCC, are presented in Table 1. The Se varied between 38.1 and 86.6%, and was highest for GM3SCC and lowest for adjusted NAGase. The Sp varied between 58.1 and 79.8%, and was highest for adjusted SCC and lowest for non-adjusted NAGase. The PPV varied between 44.1 and 66.1%, whereas NPV varied between 70.6 and 89.3%. The adjusted SCC and GM3SCC gave the highest PPV.

Figure 1. Receiver-operator characteristic (ROC) curves for SCC, lactate dehydrogenase (LDH), and N-acetyl-[β]-d-glucosaminidase (NAGase) to discern cows with IMI from those without IMI. The fourth line is the line of no-discrimination.
Figure 2. Receiver-operator characteristic (ROC) curves for SCC (adjSCC), lactate dehydrogenase (adjLDH), and N-acetyl-β-D-glucosaminidase (adjNAGase) after adjusting for different influential factors (e.g., breed, parity, season) previously found significantly associated with respective udder health indicator, to discern cows with IMI from those without IMI. The fourth line is the line of no-discrimination.

Figure 3. Receiver-operator characteristic (ROC) curves for the SCC from the test milking at bacteriological sampling (SCC525), the geometric mean of the SCC from the test milking at bacteriological sampling and the SCC from the previous adjacent test milking (GM2SCC), and the SCC from the test milking at bacteriological sampling and the SCC from the 2 previous adjacent test milkings (GM3SCC), for discerning cows with from those without IMI. The fourth line is the line of no-discrimination.
Figure 4 shows how the PPV increased, NPV decreased, and ACC slightly increased (except for the adjusted SCC) for the lnSCC, adjusted SCC, GM2SCC, and GM3SCC with increasing prevalence.

**DISCUSSION**

The results from the present study showed that SCC had a better test performance than LDH and NAGase and NPV, respectively, and the nonadjusted NAGase and adjusted NAGase gave the lowest PPV and NPV, respectively. The highest ACC (78.1) was obtained using the adjusted SCC, and the lowest ACC (59.8) was obtained using the nonadjusted NAGase.

Table 1. Sensitivity (Se), specificity (Sp), point estimate of (Se + Sp)/2, positive predictive value (PPV), negative predictive value (NPV), and accuracy (ACC) at selected cut-offs of 3 udder health indicators (UHI) to correctly classify dairy cows with or without IMI (95% CI in parentheses)

<table>
<thead>
<tr>
<th>UHI¹</th>
<th>Cut-off</th>
<th>Se</th>
<th>Sp</th>
<th>(Se + Sp)/2</th>
<th>PPV</th>
<th>NPV</th>
<th>ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>lnSCC</td>
<td>4.6</td>
<td>78.6</td>
<td>75.5</td>
<td>0.77</td>
<td>62.7</td>
<td>87.0</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>(73.1; 83.5)</td>
<td>(71.4; 79.2)</td>
<td>(0.74; 0.80)</td>
<td>(57.2; 68.0)</td>
<td>(83.5; 90.1)</td>
<td>(73.3; 79.5)</td>
<td></td>
</tr>
<tr>
<td>lnLDH</td>
<td>0.47</td>
<td>66.5</td>
<td>58.3</td>
<td>0.62</td>
<td>45.6</td>
<td>76.8</td>
<td>61.9</td>
</tr>
<tr>
<td></td>
<td>(60.4; 66.7)</td>
<td>(53.8; 62.7)</td>
<td>(0.59; 0.66)</td>
<td>(40.5; 50.8)</td>
<td>(72.2; 81.0)</td>
<td>(58.3; 65.4)</td>
<td></td>
</tr>
<tr>
<td>lnNAGase</td>
<td>1.77</td>
<td>63.0</td>
<td>58.1</td>
<td>0.61</td>
<td>44.1</td>
<td>74.9</td>
<td>59.8</td>
</tr>
<tr>
<td></td>
<td>(56.8; 69.0)</td>
<td>(53.6; 62.5)</td>
<td>(0.57; 0.64)</td>
<td>(39.0; 49.4)</td>
<td>(70.3; 79.2)</td>
<td>(56.2; 63.3)</td>
<td></td>
</tr>
<tr>
<td>Adjusted lnSCC</td>
<td>4.8</td>
<td>75.1</td>
<td>79.8</td>
<td>0.77</td>
<td>66.1</td>
<td>85.9</td>
<td>78.1</td>
</tr>
<tr>
<td></td>
<td>(69.3; 80.3)</td>
<td>(75.9; 83.2)</td>
<td>(0.74; 0.81)</td>
<td>(60.4; 71.5)</td>
<td>(82.4; 89.0)</td>
<td>(75.0; 81.1)</td>
<td></td>
</tr>
<tr>
<td>Adjusted lnLDH</td>
<td>0.60</td>
<td>56.8</td>
<td>68.1</td>
<td>0.62</td>
<td>48.3</td>
<td>75.0</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>(50.5; 63.0)</td>
<td>(63.8; 72.2)</td>
<td>(0.59; 0.66)</td>
<td>(42.6; 54.1)</td>
<td>(70.7; 79.0)</td>
<td>(60.6; 67.6)</td>
<td></td>
</tr>
<tr>
<td>Adjusted lnNAGase</td>
<td>1.94</td>
<td>38.1</td>
<td>78.1</td>
<td>0.58</td>
<td>47.8</td>
<td>70.6</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>(32.2; 44.4)</td>
<td>(74.2; 81.7)</td>
<td>(0.55; 0.62)</td>
<td>(40.8; 54.9)</td>
<td>(66.6; 74.4)</td>
<td>(60.8; 67.8)</td>
<td></td>
</tr>
<tr>
<td>GM2SCC</td>
<td>4.6</td>
<td>82.0</td>
<td>72.8</td>
<td>0.77</td>
<td>63.9</td>
<td>87.4</td>
<td>76.2</td>
</tr>
<tr>
<td></td>
<td>(75.8; 87.1)</td>
<td>(67.7; 77.5)</td>
<td>(0.74; 0.81)</td>
<td>(57.6; 69.8)</td>
<td>(82.8; 91.0)</td>
<td>(72.3; 79.8)</td>
<td></td>
</tr>
<tr>
<td>GM3SCC</td>
<td>4.3</td>
<td>86.6</td>
<td>65.6</td>
<td>0.76</td>
<td>59.6</td>
<td>89.3</td>
<td>73.3</td>
</tr>
<tr>
<td></td>
<td>(81.0; 91.1)</td>
<td>(60.2; 70.7)</td>
<td>(0.72; 0.79)</td>
<td>(53.6; 65.4)</td>
<td>(84.7; 92.9)</td>
<td>(69.3; 77.1)</td>
<td></td>
</tr>
</tbody>
</table>

¹The UHI were SCC (cells/mL), lactate dehydrogenase (LDH, U/L), and N-acetyl-β-d-glucosaminidase (NAGase, U/L). Test properties for adjusted UHI (adjusted for influential factors; for example, breed, parity, season, previously found significantly associated with respective UHI) are also presented, as well as test properties for the geometric mean of the SCC at the test milking at bacterial sampling and the SCC from 1 (GM2SCC) or 2 (GM3SCC) previous adjacent test milkings.
in detecting IMI-negative and IMI-positive cows. Almost 80% of the cows were correctly classified as positive or negative by using the adjusted or nonadjusted SCC, whereas adjusted and nonadjusted LDH and NAGase classified <65% of the IMI-positive or IMI-negative cows correctly. In other studies, however, a better agreement between SCC, LDH, and NAGase, measured in composite milk samples from cows with or without mastitis, has been found (Chagunda et al., 2006; Moyes et al., 2014). Chagunda et al. (2006) obtained higher Se (most >65%) and Sp (all >90%) for different cut-offs of LDH and NAGase compared with the present study. However, in the studies by Chagunda et al. (2006) and Moyes et al. (2014), the cows included were either clinically healthy or showing clinical signs of mastitis. This could explain some of the differences in results between those studies and our study, as the IMI-positive cows in the present study did not show any clinical signs, presumably only having subclinical mastitis. It has been shown that the correlation between SCC and LDH and that between SCC and NAGase is higher in milk samples from cows with clinical mastitis than in milk samples from clinically healthy cows (Chagunda et al., 2006). Another study found that NAGase, analyzed in quarter milk samples, was superior to SCC in identifying IMI-positive quarters in clinically healthy cows known to have chronic IMI (Mattila et al., 1986a). Even though the clinical findings of those cows were similar to that of the cows in the present study, the relationship between NAGase and SCC differed. The difference might be explained by the fact that NAGase and SCC were analyzed in composite milk samples in the present study.

Adjustment of the SCC for the influence of different cow factors (e.g., breed, DIM) has been used in Sweden since the early 1990s, is used today in Denmark, and has been discussed by others (Mattila et al., 1986b; Veclet et al., 1989; Chagunda et al., 2006). The results from the present study did not provide strong support for the use of an adjusted SCC (or adjusted LDH and NAGase) to improve test performance in finding cows with IMI as the test performance of the nonadjusted and adjusted values were very similar. However, several studies have shown that different factors (e.g., parity, season) do significantly influence SCC, LDH, and NAGase (Berning and Shook, 1992; Walsh et al., 2007; Wenz et al., 2010; Nyman et al., 2014) and veterinarians and advisors should keep this in mind if they compare SCC, LDH, and NAGase values from, for example, cows in different parities or DIM. The increase in SCC, LDH, and NAGase due to IMI is, however, generally much greater than that of other influential factors; hence, a large increase from one test milking to the next will most likely be due to an inflammatory response due to IMI and not due to influential factors such as parity or DIM.

Using information about the CMSCC from previous test milkings improved Se and NPV (while reducing Sp), especially when the geometric mean of 3 test milkings (i.e., the SCC of the test milking at bacteriological sampling and SCC from the 2 previous adjacent test milkings) was used. Thus, such an approach could give extra information in deciding if the cow is truly IMI negative based on the SCC. However, information about SCC from one test milking gives sufficient information when no other results are available (e.g., in early lactation). In accordance with our findings, Reksen et al. (2008) found no substantial effect of including SCC from more test milkings on Se, Sp, or PPV when comparing single SCC with the geometric mean SCC of up to 3 separate SCC records.

In the present study, we chose a cut-off for the calculation of test performance at which the combined Se and Sp was maximized, although from a clinical point of view this might not be optimal. Depending on the under health problem and the management and economic conditions in a specific herd, a cut-off that maximizes either Se or Sp might be needed. The SCC cut-offs used in the present study ranged between lnSCC 4.3 to 4.8 (an SCC between approximately 74,000 and 120,000 cells/mL) for the different SCC measures used, which is lower than the cut-off most commonly used (200,000 cells/mL) to classify cows as probably infected or not. Using a cut-off of 200,000 cells/mL in the present study gave a higher Sp (86.5%) but a much lower Se (55.3%) than the cut-offs chosen from the models. The Sp, when using a cut-off of 200,000 cells/mL, was very similar to findings of Dohoo and Leslie (1991; 85.5%) in a study where they, inter alia, compared Se and Sp for SCC thresholds based on various diagnostic criteria for prevalent infections. However, their Se was higher (72.6%) than ours when using a cut-off of 200,000 cells/mL in a study where they, inter alia, compared Se and Sp for SCC thresholds based on various diagnostic criteria for prevalent infections. However, their Se was higher (72.6%) than ours when using a cut-off of 200,000 cells/mL. In another study evaluating relationships between milk culture results and CMSCC, Reksen et al. (2008) found higher Sp (90.1%) but much lower Se (30.5%) than in the present study when using the 200,000 cell/mL cut-off.

In the present study, we chose to create our gold standard of cow IMI status based on bacteriological culture of milk samples from 3 consecutive samplings. This gold standard is not perfect, which can result in biases when interpreting the results. To include in the gold standard only cows classified as IMI negative or IMI positive (and not cows with inconclusive findings) may have resulted in a biased subset of infected cows, and the Sp and Se derived in this study might be biased upwards, as only very likely negative and positive cows were included. Moreover, a truly infected cow could
have been misclassified as IMI negative, even though this is not very likely when considering the criteria used. However, a truly uninfected cow would most probably not have been classified as IMI positive, given the criteria used. The Se and Sp for SCC, LDH, NAGase, adjusted values, and SCC including information from previous test milkings, however, were all affected in the same way by this bias, and comparisons between these udder health measures are therefore still valid.

When an udder health indicator is used as a diagnostic tool to find IMI-positive or IMI-negative cows, the PPV and NPV are of importance. The PPV tells us the proportion of the test-positive animals that are truly positive; for example, how many of the high SCC cows have one or more IMI positive quarters. Thus, if the PPV is low, many of the test-positive cows are not truly IMI positive. In practice, a low PPV could mean, for example, unnecessary analysis costs for the farmer if he or she wants to confirm that cows indicated to be IMI positive according to SCC truly are IMI positive. On the other hand, a low NPV indicates that many of the test-negative cows (e.g., cows with low SCC) are actually not truly IMI negative. The risk with a low NPV is that false-negative cows are not separated from true-negative IMI cows, with the risk of spreading pathogens to the truly IMI-negative cows. However, PPV and NPV are population specific, depend on the prevalence of the disease of interest in the tested population, and are not easy to compare between studies and populations. In this study, the prevalence of IMI was at least 27% (257/934) in a randomly selected, clinically healthy cow population. Hence, if SCC is used as a diagnostic tool to classify cows as IMI negative or positive on Swedish farms with a similar prevalence of IMI, our study indicates that 86 to 89% of the IMI-negative cows and 60 to 66% of the IMI-positive cows would be correctly diagnosed. In a population with higher prevalence of IMI, the PPV would increase while the NPV would decrease (Figure 4). A diagnostic tool with higher Se, Sp, PPV, and NPV than obtained by using SCC would be preferable, but considering that (1) no better diagnostic tool is available to use at test milking, (2) SCC is easy to analyze to a low price, and (3) most farmers and advisors are familiar with SCC, the use of test milking SCC is recommended as a screening tool for IMI.

CONCLUSIONS

Somatic cell count had the overall best test performance for detecting IMI-negative and IMI-positive cows compared with LDH and NAGase. The test performance of SCC, LDH, and NAGase was marginally improved by adjustments for different influential factors. Moreover, using repeated measurements of SCC slightly improved the test performance of SCC.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the Swedish Farmers’ Foundation for Agricultural Research (Stockholm, Sweden). We especially thank all the participating farmers for their hospitality and cooperation; the technicians for invaluable assistance in sampling; and the technical staff at the laboratory at the Department of Animal Health and Antimicrobial Strategies (SVA, Uppsala, Sweden), the Department of Bacteriology (SVA, Upplands, Sweden), and the Department of Animal Science (Aarhus University, Tjele, Denmark). We also want to give our gratitude to Torben W. Bemmedsgaard and Torben Larsen at the Department of Animal Science (Aarhus University, Tjele, Denmark) for contributing knowledge about, and analysis of, LDH and NAGase.

REFERENCES


Mattila, T., J. Syvajärvi, and M. Sandholm. 1986b. Milk antitrypsin, NAGase, plasmin and bacterial replication rate in whey: Effects of...


APPENDIX

Here we present the equations used to adjust the udder health indicators SCC, LDH, and NAGase for influential factors previously found significantly associated with respective udder health indicator (Nyman et al., 2014).

SCC

As the Box-Cox transformed SCC (bcSCC) was used in the models reported by Nyman et al. (2014), bcSCC was first adjusted for cow factors and then back transformed to an adjusted SCC that was used for the transformations/categorizations of SCC used in the present study. The bcSCC was adjusted for parity, breed, milk yield, percentage of milk fat, and the interaction between breed and milk urea concentration according to the following equation:

$$bcSCC + (0.383 \times \text{parity} 1) + (0 \times \text{parity} 2) - (0.045 \times \text{parity} 3) - (0.264 \times \text{parity} 4) - (0.496 \times \text{parity} 5) + [(0.240/2) \times SR] - [(0.240/2) \times SH] - (0.017 \times \text{milk yield}) - (0.137 \times \text{milk fat}) - [(-0.015 \times \text{milk urea}) \times SR] - [(-0.015 \times \text{milk urea}) + (0.094 \times \text{milk urea})] \times SH),$$

and all continuous variables were centered at their mean.

LDH

Log-transformed LDH (lnLDH) was adjusted for parity, DIM, percentage of milk protein, milk urea concentration, and season according to the following equation:

$$\lnLDH + (0.187 \times \text{parity} 1) + (0 \times \text{parity} 2) + (0.054 \times \text{parity} 3) - (0.083 \times \text{parity} 4) - (0.316 \times \text{parity} 5) - (0.062/100) - (0.219) - (0.230 \times \text{milk protein}) - (0.089 \times \text{milk urea}) + \{(0.217+0.184)/2 \times \text{season0}\},$$

and all continuous variables were centered at their mean, and DIM was scaled (DIM/100).

NAGase

Log-transformed NAGase (lnNAGase) was adjusted for parity, DIM, milk yield, milk urea concentration, and season according to the following equation:

$$\lnNAGase + (0.164 \times \text{parity} 1) + (0 \times \text{parity} 2) - (0.038 \times \text{parity} 3) - (0.097 \times \text{parity} 4) - (0.139 \times \text{parity} 5) - (0.183/100) - (0.022 \times \text{DIM}^2) - (0.006 \times \text{milk yield}) - (0.073 \times \text{milk urea}) + \{(0.173+0.131)/2 \times \text{season0}\},$$

and all continuous variables were centered at their mean, and DIM was scaled (DIM/100).