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Associations of udder-health indicators with cow factors and with intramammary infection in dairy cows

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ABSTRACT

The objective of this study was to investigate if and how cow factors and intramammary infection (IMI) are associated with 4 different udder-health indicators in dairy cows as a first step in investigating whether the diagnostic performance of these indicators can be improved. The investigated indicators were somatic cell count (SCC), lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAGase), and alkaline phosphatase (AP) measured in milk. In this cross-sectional study, approximately 1,000 cows from 25 dairy herds were sampled for bacteriology (quarter milk samples) during 3 consecutive days: the day before test milking, at the day of test milking, and at the day after test milking. The whole-udder test milking sample was analyzed for milk composition, SCC, LDH, NAGase, and AP. Cow data (parity, breed, milk yield, percentage of milk fat and protein, milk urea concentration, and days in milk from the sampled test milking) were collected from the Swedish milk-recording scheme. Of the sampled cows 485 were considered IMI negative and were used in multivariable mixed-effect linear regression models to investigate associations between cow factors and the udder-health indicators. A second modeling including all cows, both IMI negative and IMI positive (256 cows), was also performed. The results showed that all udder-health indicators were affected by cow factors but that different cow factors were associated with different indicators. Intramammary-infection status was significantly associated with all udder-health indicators except AP. Parity and milk urea concentration were the only cow factors associated with all indicators in all models. The significant cow factors explained 23% of the variation in SCC and >30% of the variation in LDH, NAGase, and AP in IMI-negative cows, showing that LDH, NAGase, and AP are more affected than SCC by cow factors. The IMI status explained 23% of the variation in SCC in the model with all cows but only 7% of the variation in LDH and 2% of the variation in NAGase, indicating that SCC has the best potential as a diagnostic tool in finding cows with IMI. However, further studies are needed to investigate whether the diagnostic properties of these udder-health indicators will improve with adjustment according to their associations with different cow factors when used as a diagnostic tool for finding cows with IMI.
Key words: somatic cell count, lactate dehydrogenase, N-acetyl-β-D-glucosaminidase, alkaline phosphatase

INTRODUCTION

Mastitis in dairy cows causes economic losses for the farmers (Halasa et al., 2007) because of reduction in milk production (Hagnestam et al., 2007; Dürr et al., 2008) and milk quality (Kitchen, 1981; Sandholm et al., 1995), costs of veterinary services, extra labor, and so on, and it affects the welfare of the cow. Cases of clinical mastitis are generally easy to detect because the signs are visible, and the affected cow can be treated and separated from healthy cows, eliminating a risk of transition of udder pathogens to healthy cows. Cows with subclinical mastitis, however, have no visible signs of mastitis and are therefore a hidden threat to healthy cows in the herd. In Sweden the most common udder pathogen found in cases of subclinical mastitis is *Staphylococcus aureus* (Persson et al., 2011), a pathogen considered to be very contagious; hence, it is very important to identify infected cows to reduce the spread of bacteria in the herd. This reasoning is also valid for other contagious udder infections.

Today the best method to identify cows with IMI is to take milk samples for bacterial culturing or PCR assay. However, to sample all udder quarters of all cows in a herd is expensive. Instead, diagnostic tools identifying the inflammatory response are used, most commonly involving analyses of SCC in composite milk samples (from the test milking), because bacterial infections generally lead to increased SCC. Milk samples from high-SCC cows can then be collected and analyzed for

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bacterial content. However, other factors than IMI have been shown to affect the SCC, e.g., parity (Walsh et al., 2007; Reksen et al., 2008), breed (Brolund, 1985; Walsh et al., 2007), DIM (Brolund, 1985; Laevens et al., 1997; Schepers et al., 1997), and milk production (Brolund, 1985; Walsh et al., 2007), which may reduce the specificity (**Sp**) and the negative predictive value of SCC as a diagnostic tool, especially if a low SCC cut-off is used. Hence, to improve the diagnostic abilities of SCC, the test milking composite milk SCC (**CMSCC**) can be adjusted according to the associations with different cow factors. This adjustment is meant to make it easier to identify cows with an increase in SCC due to IMI, because it would increase both the sensitivity (**Se**) and Sp of SCC, and to make it easier to compare the CMSCC of cows in a herd. In Sweden, the CMSCC has been adjusted, since the late 1980s, for parity, breed, milk yield (kg of milk/d), and DIM, and the adjusted CMSCC of 2 to 3 consecutive test milkings is used in a regression analysis to calculate a probability that the cow has one or more udder quarters with IMI (Brolund, 1990). This tool is called udder-health classes. Udder-health classes are used by farmers to group cows in "probably infected" and "probably uninfected" and as a tool to select cows for sampling for bacteriology and for dry-cow therapy.

Udder-health indicators other than SCC exist, which also change during inflammation and can be measured in milk. The activity of the enzymes lactate dehydrogenase (**LDH**), N-acetyl-β-D-glucosaminidase (**NAGase**), and alkaline phosphatase (**AP**) have all been shown to be associated with mastitis or IMI (Bogin and Ziv, 1973; Nielsen et al., 2005; Chagunda et al., 2006; Babaei et al., 2007). These udder-health indicators have not yet been able to replace SCC as the most-used udder-health measurement tool. However, in recent years new and more efficient and economically viable analysis methods of the activity of these enzymes have been developed (Larsen, 2005; Larsen et al., 2010). Some studies have shown that these enzymes also are affected by cow factors, but they do not show to what extent (Berning and Shook, 1992; Chagunda et al., 2006; Piccinini et al., 2007; Wenz et al., 2010).

The need for adjustment of SCC, LDH, and NAGase for the effect of different cow factors has been discussed by others (Mattila et al., 1986; Vecht et al., 1989; Chagunda et al., 2006). However, if adjustments of CMSCC are used, the underlying calculations of the adjustments need to be revised regularly as the cow population changes genetically, and associations found decades ago may not be applicable to the cows of today. Hence, the main aim of the present project was to investigate if and how different cow factors and CMSCC, LDH, NAGase, and AP are associated in IMI-negative cows. Our hypotheses were that CMSCC in IMI-negative cows are affected by cow factors and that LDH, NAGase, and AP activities in IMI-negative cows also are affected by cow factors but to a lower extent than SCC. A model including only IMI-negative cows would more clearly show the systematic effects of the cow factors on each udder-health indicator compared with a model including all cows where IMI status can have a substantial confounding effect with the other explanatory variables. A second aim was to investigate how IMI status was associated with the different udder-health indicators to assess whether some of the indicators have better potential as a diagnostic tool to distinguish cows with and without IMI.

MATERIALS AND METHODS

Study Design

This study was designed as a cross-sectional study. The sample size was calculated to obtain a good estimate of the Se/Sp of CMSCC when used as a diagnostic tool to predict cows as IMI negative and IMI positive. To obtain a Se/Sp of 0.8 with a precision of 10%, a 95% confidence interval, and a cluster effect of 3, at least 183 observations would be needed. With an estimated prevalence of IMI of 20%, approximately 1,000 observations would be needed to be able to get the desired Se/Sp. A random sample of herds (n = 35) with a milking parlor, an annual herd size of 60 to 200 dairy cows, at least 30% of the cows of each of the 2 main Swedish dairy breeds (Swedish Red, **SR**, and Swedish Holstein, **SH**), and an estimated bulk milk SCC of 150,000 to 300,000 cells/mL were contacted and asked to participate. In total, 25 dairy herds located in the southern half of Sweden were recruited, and to obtain a total of 1,000 sampled cows, 40 cows were sampled in each herd.

Visits

Each herd was visited twice, 2 to 4 mo apart, during the housing season, i.e., between October 2009 and April 2010. At these visits, a technician from the local livestock association attended one milking occasion (morning or evening) at the day before the routine test milking, at the day of test milking, and at the day after test milking. The main author met all technicians before the study started and informed them about the sampling technique used in the study.

Cow Selection

Because the aim of the project was to investigate associations between udder-health indicators and cow fac-