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Test characteristics of milk amyloid A ELISA, somatic cell count, and bacteriological culture for detection of intramammary pathogens that cause subclinical mastitis

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ABSTRACT

Bovine mastitis is an important disease in the dairy industry, causing economic losses as a result of withheld milk and treatment costs. Several studies have suggested milk amyloid A (MAA) as a promising biomarker in the diagnosis of mastitis. In the absence of a gold standard for diagnosis of subclinical mastitis, we estimated the diagnostic test accuracy of a commercial MAA-ELISA, somatic cell count (SCC), and bacteriological culture using Bayesian latent class modeling. We divided intramammary infections into 2 classes: those caused by major pathogens (e.g., *Escherichia coli*, *Staphylococcus aureus*, streptococci, and lacto-/enterococci) and those caused by all pathogens (major pathogens plus *Corynebacterium bovis,* coagulase-negative staphylococci, *Bacillus* spp., *Streptomyces* spp.). We applied the 3 diagnostic tests to all samples. Of 433 composite milk samples included in this study, 275 (63.5%) contained at least 1 colony of any bacterial species; of those, 56 contained major pathogens and 219 contained minor pathogens. The remaining 158 samples (36.5%) were sterile. We determined 2 different thresholds for the MAA-ELISA using Bayesian latent class modeling: 3.9 μg/mL to detect mastitis caused by major pathogens and 1.6 μg/mL to detect mastitis caused by all pathogens. The optimal SCC threshold for identification of subclinical mastitis was 150,000 cells/mL; this threshold led to higher specificity (Sp) than 100,000 cells/mL. Test accuracy for major-pathogen intramammary infections was as follows: SCC, sensitivity (Se) 92.6% and Sp 72.9%; MMA-ELISA, Se 81.4% and Sp 93.4%; bacteriological culture, Se 23.8% and Sp 95.2%. Test accuracy for all-pathogen intramammary infections was as follows: SCC, sensitivity 90.3% and Sp 71.8%; MAA-ELISA, Se 88.0% and Sp 65.2%; bacteriological culture, Se 83.8% and Sp 54.8%. We suggest the use of SCC and MAA-ELISA as a combined screening procedure for situations such as a *Staphylococcus aureus* control program. With Bayesian latent class analysis, we were able to identify a more differentiated use of the 3 diagnostic tools. The MAA-ELISA is a valuable addition to existing tools for the diagnosis of subclinical mastitis.
Key words: subclinical mastitis, somatic cell count, milk amyloid A, bacteriological culture, Bayesian latent class

INTRODUCTION

Subclinical mastitis is difficult to detect because of a lack of clinical signs that can be easily identified by visual inspection and palpation of the udder. Reliable diagnostic methods are needed to detect subclinical mastitis. In Swiss veterinary practice, the diagnosis of subclinical mastitis is based on the results of a California Mastitis Test, SCC, or bacteriological culture (**BC**). However, it has been postulated that the California Mastitis Test and SCC are not sensitive enough to identify subclinical infection, and do not qualify as reliable screening tests (Middleton et al., 2004; Safi et al., 2009). As well, SCC usually remains elevated for several weeks after successful treatment (Pyörälä, 1988) and is affected by many physiological factors, such as age, lactation period, parity, stress, season, and intra- or extramammary infection (Jensen and Eberhart, 1981; Bielfeldt et al., 2004; Sharma et al., 2011). Furthermore, the amplitude of the increase in SCC depends on the pathogen. Minor pathogens (e.g., *Corynebacterium bovis*, CNS) show less of an effect on SCC than major pathogens (e.g., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae,* and *Streptococcus uberis*; Djabri et al., 2002). To evaluate diagnostic tests for IMI, most recent studies have applied single-sample BC as the gold standard, despite observations that it is affected by contamination and intermittent bacterial shedding, resulting in a low sensitivity (**Se**; Sears et al., 1990). The suitability of BC as a gold standard has

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been questioned (Grönlund et al., 2005; Andersen et al., 2010) unless 3 consecutive milk samples are used (Dohoo et al., 2011); a referential diagnostic procedure with higher Se and specificity (**Sp**) is desirable.

A promising approach may be to measure the concentration of acute-phase proteins in milk samples. The acute-phase reaction is part of the innate immune system triggered by challenges such as infection, inflammation, or stress (Grönlund et al., 2005). Unlike in humans, dogs, and pigs, where C-reactive protein makes up most acute-phase proteins (Eckersall, 2000; Eckersall et al., 2006), in cattle the major acute-phase proteins are haptoglobin and serum amyloid A (McDonald et al., 2001; Grönlund et al., 2003; Akerstedt et al., 2007; Kovac et al., 2011). An elevated serum concentration of haptoglobin or serum amyloid A is a nonspecific marker for inflammation anywhere in the animal; it must be present in milk to provide relevant information about udder health (Eckersall et al., 2001). Serum amyloid A migrates passively through the blood-milk-barrier because of the increased permeability of inflamed mammary tissue (Eckersall et al., 2001; Kovac et al., 2011), and it is also locally produced as a particular isoform (**M-SAA3**) by a restricted population of bovine mammary epithelial cells (McDonald et al., 2001; Gerardi et al., 2009; Molenaar et al., 2009). Serum amyloid A and M-SAA3 together are called milk amyloid A (**MAA**), which is measurable in milk samples using a commercially available ELISA (Tridelta Development Ltd., Maynooth, Ireland), and MAA has proven to be a reliable biomarker for both subclinical mastitis (Eckersall et al., 2006; Gerardi et al., 2009; Safi et al., 2009; Pyörälä et al., 2011) and clinical mastitis (Molenaar et al., 2009; Kovac et al., 2011; Pyörälä et al., 2011).

As mentioned above, single-sample BC as a gold standard, with sensitivity <100%, is ill suited to evaluate the accuracy of new tests, and tends to underestimate their specificity. An alternative approach is to estimate diagnostic accuracy with Bayesian latent class modeling, using results from at least 3 different diagnostic tests of the same sample (Branscum et al., 2005; Berkvens et al., 2006; Hartnack et al., 2013). The aim of this study was to estimate the diagnostic Se and Sp of SCC, MAA-ELISA, and BC for the diagnosis of subclinical mastitis in dairy cows using Bayesian latent class modeling.

MATERIALS AND METHODS

Animals and Samples

For the present study, 435 composite milk samples were collected between April and July 2015 as part of a *Staph. aureus* control program for selected mountain summer pastures in a veterinary practice in Scuol, Switzerland (Clinica Alpina, 5 veterinarians). Only lactating cows with macroscopically healthy udders (i.e., no pathological findings by visual inspection and palpation) were included in the study. All cows from 1 farm that were designated to join the summer mountain pasture program were sampled on the same day. Milk samples were collected aseptically after disinfection of the teat with 70% alcohol on a cotton ball. The first 3 squirts from each quarter were discarded before milking 1 squirt from each teat into the same sterile tube. Samples were taken during the daily veterinary routine and transported at room temperature for 0.5 to 8 h (with random distribution) before BC and SCC at the laboratory in the veterinary practice. Then, samples were stored at −18°C until the MAA assay was performed. The sampled cows belonged to 39 farms, were in different lactation periods (1 to 20 mo after calving, median 6 mo), and were in different parities (first to eleventh lactation, median second lactation). Cows ranged from 1 to 20 yr of age (median 5.5 yr).

Laboratory Procedures

Bacteriological Culture. The BC were performed by 2 laboratory assistants at Clinica Alpina. Approximately 0.01 mL of each sample was streaked on trypticase soy agar with sheep blood and on MacConkey agar No.3 (**MC3**; Oxoid, Basingstoke, United Kingdom). Agar plates were evaluated after 24 h of incubation at 37°C, and a first suspicion was determined according to the morphological characteristics of the colonies on trypticase soy agar with sheep blood and MC3, as well as by chemical reactions, such as catalase reaction with H_2O_2 and Gram stain. Suspicions were confirmed with a second reading 24 h later. Staphylococci without hemolysis on the trypticase soy agar with sheep blood were classified as CNS, and colonies with αβ- or only β-hemolysis were classified as *Staph. aureus*. Because α-hemolytic staphylococci can represent CNS or *Staph. aureus*, 1 of these colonies was transferred to a chromogen agar (chromID *Staph. aureus* agar; BioMérieux, Marcy-l'Étoile, France). This agar identifies *Staph. aureus* using a green coloration of the colonies depending on the production of α-glucosidase after 18 to 20 h of incubation. It has Se of 96.8% and Sp of 97.4% and was set up with a *Staph. aureus*–positive control (Perry et al., 2003). Only colonies showing the same phenotype as the positive control were classified as *Staph. aureus*; colonies with a different phenotype were classified as CNS. Streptococci were further classified as *Strep. uberis, Strep. dysgalactiae* and *Strep. agalactiae* by esculin reaction (EscTSASB; Oxoid) and using the