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Differential cell count as an alternative method to diagnose dairy cow mastitis

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ABSTRACT

Changes in relative cell proportions occurring in diseased mammary glands of dairy cows can be determined using differential cell count (DCC). The present study was carried out in 2 consecutive trials, with 2 goals: (a) to test the consistency of DCC results on subsequent days, and (b) to establish an effective cutoff value for the diagnosis of mastitis. In the first trial, quarter milk and blood samples were taken from 8 healthy cows for 5 consecutive days. Milk samples were tested by somatic cell count (SCC) and bacteriological analysis, and DCC was performed on blood and milk samples by flow cytometer. In the second trial, 16 animals were randomly selected from a different herd and quarter milk samples taken on 3 consecutive milkings. All samples were cyto-bacteriologically analyzed and DCC was performed on the second sampling. In the first trial, mean SCC was 77,770 cells/mL and 4 samples were bacteriologically positive. No fixed or random effect had a significant influence on percentages of individual cell populations or ratios in blood or milk. A cutoff value of 0.495 for logarithmic polymorphonuclear neutrophilic leukocyte:lymphocyte ratio was established. Mean SCC of milk samples collected in the second trial was 543,230 cells/mL, and infection was detected in 53.1% of quarters, mostly caused by *Staphylococcus aureus*. When the cutoff value was applied to the data along with SCC, sensitivity and specificity of the diagnostic method were 97.3 and 92.3%, respectively.
Key words: dairy cow, differential cell count, diagnosis, subclinical mastitis

INTRODUCTION

Subclinical mastitis is a major health problem in dairy cattle. Economic losses are mostly associated with decreased production and milk quality. Such infections are not evident and can persist in the mammary tissue throughout lactation. *Staphylococcus aureus* is a contagious pathogen and a major agent of subclinical mastitis (IDF, 2006), but the infection can also be caused by a wide range of environmental and opportunistic pathogens (Bradley, 2002).

Subclinical mastitis can be diagnosed by SCC, bacteriological analysis, or PCR. The International Dairy Federation recommends the use of both SCC and bacteriological analysis for the determination of udder health (Hogan et al., 1999). Accordingly, the diagnosis of bovine mastitis is mostly based on cyto-bacteriological analysis of milk samples (Vangroenweghe et al., 2002). Despite that, identification of infected quarters presents difficulties related to the possibility of false-negative bacteriological results and infections without a concomitant increase of SCC (Schwarz et al., 2010).

Differential cell count (**DCC**) shows changes in relative cell proportions, which can be used to differentiate healthy glands from inflamed or infected glands, and DCC has been proposed as a valid tool for the identification of inflammatory processes in cases with low SCC (Rivas et al., 2001). Recent studies (Schwarz et al., 2011a,b; Pilla et al., 2012) have shown that DCC can reveal inflammatory processes, even in milk with SCC of 1,000 cells/mL, well below the current threshold of 100,000 cells/mL (DVG, 2002).

Differential cell count can be performed using different methods. Microscopic DCC is a simple and cost-effective method, but most researchers prefer cytometric analysis because of its higher accuracy. Leitner et al. (2000b) found a high correlation between the 2 methods for PMNL and lymphocytes, but a lower correlation for macrophages and epithelial cells, probably because of the difficulty in differentiating between these cell populations with light microscopy. Different cell patterns have been documented during the course of infection in the presence of different pathogens (Leitner et al., 2000b). In acute mastitis, PMNL are the predominant cell type, often accounting for more than 90% of the total mammary leukocyte population (Sordillo and Streicher, 2002). In contrast, in chronic mastitis caused by *Staph. aureus* and CNS, PMNL percentages can vary from the high values seen in acute mastitis to per-

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centages as low as those recorded in uninfected quarters (Leitner et al., 2000b, 2003; Riollet et al., 2001). The effect of lactation stage and parity number should also be taken into account. Lymphocytes and monocytes are reported to be higher in early lactation than in mid and late lactation, whereas macrophages and PMNL percentages are considerably lower (Dosogne et al., 2003).

Lymphocytes, macrophages, and PMNL play an important role in the immunity of the mammary gland (Paape et al., 1979; Sordillo and Nickerson, 1988). A successful defense against invading pathogens depends on number and distribution of leukocytes (Leitner et al., 2003). In healthy milk, the percentage of each cell type is widely variable; according to some researchers, macrophages are the predominant cell type (Riollet et al., 2001; Lindmark-Mansson et al., 2006), whereas others have shown that lymphocytes are the major population (Park et al., 1992; Leitner et al., 2000a; Schwarz et al., 2011a,b). Leitner et al. (2000a) demonstrated a high repeatability for samples taken from the same cow in different stages of lactation and suggested that the leukocyte pattern in uninfected mammary glands is genetically controlled. To the best of our knowledge, however, no information on short-term repeatability is available. Because the immune system is dynamic and the mammary gland is subjected to persistent stress during lactation, a basic knowledge of the cellular profile in healthy glands is fundamental. Therefore, the goals of the present study were (a) to investigate DCC in milk from healthy mammary quarters and to test whether the results are consistent on subsequent days; and b) to establish an effective cutoff value for the diagnosis of mastitis that is applicable under field conditions. The study was carried out in 2 consecutive trials, the first to determine DCC stability and cutoff and the second to test this cutoff value under field conditions.

MATERIALS AND METHODS

Animals and Milk Sampling

Trial 1. To investigate DCC in healthy quarters and its test–retest reliability, the herd enrolled in the first trial was located in the Lombardy region of Italy, and was certified free of paratuberculosis, bovine viral diarrhea, and infectious bovine rhinotracheitis; it also had no history of contagious mastitis pathogens in the last 10 yr. The herd consisted of 50 lactating Holstein-Friesian dairy cows housed in freestalls and milked twice daily in a milking parlor.

Eight cows were selected based on low SCC and 2 negative results of bacteriological analysis in the week before samplings. Of these, 3 cows were primiparous, 4 were in the second or third lactation, and 1 had calved 4 times. Two animals were in early lactation (83 to 111 DIM), 3 were in mid lactation (144 to 172 DIM), and 3 in late lactation (233 to 357 DIM).

Blood and quarter milk were sampled on 5 consecutive days at morning milking. All cows were free of clinical signs of mastitis at sampling. After cleaning and disinfection of the teat, the first squirts of milk were discarded, and 250 mL of milk was aseptically collected from each quarter into sterile plastic tubes (Falcon, BD Biosciences, Franklin Lakes, NJ) for both bacteriological and DCC analysis. Blood samples (10 mL) were collected by tail venipuncture into commercial EDTA-containing evacuated tubes (Vacutainer, BD Biosciences, San Jose, CA). Samples were refrigerated until arrival at laboratory facilities.

Trial 2. The calculated cutoff value was tested under field conditions in another herd located in Lombardy that was participating in a voluntary control program for contagious mastitis. The herd consisted of 180 lactating Holstein-Friesian dairy cows housed in freestalls and milked twice daily in a milking parlor. The herd had a history of high prevalence of *Staph. aureus* (approximately 50% prevalence at the beginning of the control program), and mammary infections caused by *Prototheca zopfii* had recently been detected.

In total, 16 cows were randomly selected from the last milking group, which included animals previously diagnosed as infected with *Staph. aureus* or *P. zopfii* and other animals before culling. Of these, 9 cows were primiparous and 7 multiparous.

Quarter milk samples for bacteriological analysis were collected at 3 consecutive milkings. After cleaning and disinfection of the teat, the first 2 squirts of milk were discarded, and 10 mL of foremilk was aseptically collected in sterile plastic tubes (Bioster, Seriate, Italy). At the second milking, an additional 200 mL of quarter milk was sampled for DCC analysis. Samples were refrigerated until arrival at laboratory facilities.

SCC and Bacteriological Analysis

All samples were submitted to bacteriological analysis, which was performed as previously described (Oliver et al., 2004). Briefly, an aliquot of 10 μL of each sample was spread onto blood agar plates (5% bovine blood, Oxoid, Basingstoke, UK) and plates were incubated at 37°C. Plates were evaluated after 24 and 48 h, and colonies of growth were isolated. All colonies were identified by biochemical tests following Hogan et al. (1999). Somatic cells were counted on a Bentley Somacount 150 (Bentley Instruments, Chaska, MN).

DCC

Differential cell counts were performed on blood samples and quarter milk samples by cytometry. Milk cells

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