J. Dairy Sci. 96:6336–6346
http://dx.doi.org/10.3168/jds.2013-6631

Presence of viral and bacterial organisms in milk and their association with somatic cell counts

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ABSTRACT

About 20 to 35% of milk samples from cows with intramammary infection or high somatic cell count (SCC) are negative on bacteriological culture analysis. However, little is known about SCC in milk of cows infected with viruses. In the first part of our study, we developed a real-time PCR assay for detection of bovine herpesvirus (BHV) 1, BHV2, and BHV4, and bovine viral diarrhea virus (BVDV) in composite quarter milk samples. A total of 1,479 lactating cows of 1,964 cows in the dairy herd were initially selected because these cows had complete SCC data for at least 3 consecutive test results, of which 139 lactating cows from different lactation age groups were selected randomly and studied extensively. Composite quarter milk samples were collected on 3 alternate days and examined for viruses, SCC, and bacteriological analysis. In total, 10, 28, and 0.7% of the composite quarter milk samples from cows were positive for BHV1, BHV2, and BHV4, respectively; BVDV was not detected in composite quarter milk samples. Bovine herpesvirus was not associated with a particular bacterial species. Our study results indicate that cows positive for BHV in composite quarter milk samples alone are less likely to have elevated SCC compared with cows with bacterial intramammary infection; BHV1, BHV2, and BHV4 are probably not major udder pathogens.
Key words: bovine mastitis, bovine herpesvirus, somatic cell count, real-time PCR

INTRODUCTION

Mastitis is the inflammation of the mammary gland caused by microorganisms or trauma to the udder. During the inflammatory response, the immune system of the mammary gland is activated to eliminate the pathogen (Oviedo-Boyso et al., 2007). The number of somatic cells secreted in milk, referred to as SCC, is an indicator of the inflammatory response to an IMI (Schukken et al., 2003). Somatic cell count thresholds ranging from 100,000 to 300,000 cells/mL have been used to classify uninfected (<100,000 cells/mL) and infected (>300,000 cells/mL) quarters (Deluyker et al., 2005; Berry and Meaney, 2006; Schwarz et al., 2010). In mastitis prevention and control practices, an operational threshold of 200,000 cells/mL is typically used to reduce the diagnostic error to prevent misclassifying an udder with IMI (Schukken et al., 2003).

Mastitis-causing organisms have been categorized as contagious or environmental based on their distinct characteristics of distribution and interaction with the teat and teat duct (Smith and Hogan, 1993). In addition to *Chlamydia* and *Prototheca*, nonbacterial pathogens such as fungi and yeast have also been reported to cause bovine mastitis (Radostits et al., 2000; Roesler et al., 2001). Bacteriological culture analysis is the standard method for identifying IMI. However, culture of quarter milk samples of cows with subclinical mastitis often yields no pathogens (Sargeant et al., 2001). Despite intensive etiological research, around 20 to 35% of clinical cases of bovine mastitis have an unknown etiology (Wellenberg et al., 2002b).

Wellenberg et al. (2002b) reviewed the role of viral infections in bovine mastitis. They observed that under natural cases of bovine mastitis, bovine herpesvirus 1 (**BHV1**), bovine herpesvirus 4 (**BHV4**), foot and mouth disease (**FMD**) virus, and parainfluenza type 3 (**PI3**) virus have been isolated from milk. Bovine herpesvirus 1, FMD, and PI3 viral infections caused clinical mastitis, whereas BHV4 infection caused subclinical mastitis. Viruses such as bovine herpesvirus 2 (**BHV2**), cowpox, pseudocowpox, FMD, vesicular stomatitis virus, and papillomavirus cause teat lesions. These viruses may damage the integrity of the udder and indirectly contribute to mastitis. It is probable that virus-induced immunosuppression underlies mastitis but experimental evidence to support this assumption is lacking. Very few epidemiological studies support a

Received January 28, 2013.
Accepted July 4, 2013.
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causal relationship between viral infections and bovine mastitis in field conditions (Wellenberg et al., 2002b).

The aim of this study was to determine if such an association between the presence of select viral etiological agents, including BHV1, BHV2, BHV4, and bovine viral diarrhea virus (**BVDV**) and elevated somatic cells (>200,000 cells/mL of milk) in composite quarter milk samples in the absence of bacterial IMI.

MATERIALS AND METHODS

Study Population

A large dairy herd in central Pennsylvania consented to participate in the study. The DHIA computerized data set for milk SCC for 1,964 cows in this herd was analyzed. A total of 1,798 lactating cows had SCC data, of which 1,479 lactating cows (101, 605, 552, and 221 cows in lactations 1, 2, 3, and ≥4, respectively) had SCC information for at least 3 sampling test periods. Using a random number generation technique, 10% of the cows (n = 148, including 10, 61, 55, and 22 cows in lactations 1, 2, 3, and ≥4, respectively) were selected for the study. During the course of sample collection, 9 cows left the study (5, 2, 1, and 1 cows in lactations 1, 2, 3, and ≥4, respectively) as they were either segregated from the milking herd, culled, or dried off, resulting in complete set of samples from 139 lactating cows (5, 59, 54, and 21 lactating cows in lactations 1, 2, 3, and ≥4, respectively) for analysis. Milk samples from 139 cows were examined for SCC, bacteriological analysis, and detection of BHV1, BHV2, BHV4, and BVDV by real-time PCR. The 139 cows at the time of sampling were between 90 and 120 DIM. In this herd, all adult cows are vaccinated at the time of drying off using killed vaccine against BHV-1 virus (infectious bovine rhinotracheitis, **IBR**), BVDV, PI3, and bovine respiratory syncytial virus (**BRSV**); at the time of breeding, modified live IBR, BVD, PI3, and BRSV were used.

Collection of Milk Samples

Composite quarter milk samples were collected aseptically according to National Mastitis Council guidelines (National Mastitis Council, 1990). Just before milking, teats were carefully cleaned using cotton wool dipped in 70% ethanol. The first few streaks of milk were discarded and approximately 3 mL of milk was collected from each mammary gland quarter into a sterile 15-mL screw-cap centrifuge tube. Milk was collected before morning milking in the milking parlor. Composite quarter milk samples were collected from each of the 139 cows in the study on d 1, 3, and 5. All milk samples were shipped on ice to the laboratory within 2 to 3 h of collection. Milk samples were processed on the same day of collection for determination of SCC and bacteriological analysis. The remaining milk samples were stored in 2-mL Eppendorf tubes at −20°C for subsequent analysis.

Determination of SCC in Composite Quarter Milk Samples

Each milk sample was mixed thoroughly by inverting the tube several times. The milk sample (60 μL) was drawn into a milk cassette and SCC was estimated using a DeLaval somatic cell counter (DeLaval Inc., Kansas City, MO).

Bacteriological Culture Analyses of Composite Quarter Milk Samples

The composite quarter milk samples were examined for the presence of contagious and environmental mastitis pathogens as described previously (Jayarao et al., 2004). Partitioned Petri dishes (quad plates) were used for bacterial isolation. Each quadrant of the Petri dish contained the following media, respectively: (1) Edwards modified medium (Oxoid, Basingstoke, UK) with colistin sulfate and oxolinic acid medium (**EMCO**; Sawant et al., 2002) for isolation and presumptive identification of *Streptococcus agalactiae* and environmental streptococci; (2) MacConkey's agar (Oxoid) for isolation of coliform and non-coliform gram-negative bacteria; (3) Baird-Parker agar (**BPA**; Difco, Becton Dickinson, Sparks, MD) for isolation of *Staphylococcus aureus* and CNS; and (4) 5% sheep blood agar for detection of hemolytic bacteria. The milk samples were mixed thoroughly by gently inverting the tubes several times. A loopful (~10 μL) of raw milk was streaked onto each medium and the quad plates were incubated at 37°C for 48 h. Colonies on EMCO medium were selected and streaked on 5% sheep blood agar and incubated for 48 h at 37°C. All isolates were examined for Gram reaction and catalase production, and were identified using API 20 Strep (BioMerieux, Hazelwood, MO).

Colonies suggestive of *Staph. aureus* and CNS on BPA were selected for speciation. Selected colonies were streaked on 5% sheep blood agar and incubated for 48 h at 37°C. The isolates were examined for hemolysis, coagulase test, and catalase production and identified using API-Staph (BioMerieux; National Mastitis Council, 1999).

Based on colony morphology and lactose fermentation on MacConkey's agar (Oxoid), isolates were selected for bacterial identification. Gram stain and biochemical tests such oxidation-fermentation test, oxidase test,