J. Dairy Sci. 97:4876–4885
http://dx.doi.org/10.3168/jds.2013-7657

Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle

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ABSTRACT

This study was conducted to evaluate the association between subclinical intramammary infection (IMI) with coagulase-negative staphylococci (CNS), mammary quarter milk somatic cell count (SCC), and persistence of IMI in dairy cattle. Convenience samples of CNS isolates harvested from milk samples of subclinically infected mammary quarters collected between 4 and 2 wk before drying-off, between 2 wk before drying-off and the day of drying-off, within 24 h after calving, between 1 and 2 wk after calving, and during lactation were evaluated. Isolates were obtained from the Canadian Bovine Mastitis Research Network culture bank and were identified to the species level using *rpo*B gene sequencing. Cow and quarter-level data were obtained from the Canadian Bovine Mastitis Research Network database and used for statistical analyses. In addition, for mammary quarters that had more than one isolation of the same CNS species at different time points, the isolates were evaluated using pulsed-field gel electrophoresis to identify persistent IMI. Milk SCC was compared between mammary quarters infected with different CNS species and to a cohort of uninfected mammary quarters. A total of 877 isolates from 643 mammary quarters of 555 cows on 89 Canadian dairy farms were identified to the species level. Twenty different species were identified, with *Staphylococcus chromogenes* being the most common species identified (48% of isolates), followed by *Staphylococcus simulans* (19%) and *Staphylococcus xylosus* (10%). Of the 20 species identified, only 9 species were found in persistently infected quarters. Milk SCC was significantly higher in the CNS-infected mammary quarters than in the uninfected control quarters for 8 of the 20 species studied. Also, mean SCC differed significantly between mammary quarters infected with different CNS species. Within a given species, a high degree of variability was noted in milk SCC. These data corroborate recent data from Europe with regard to the predominance of certain species of CNS (e.g., *Staph. chromogenes*). In addition, some species of CNS appear to have a greater effect on milk SCC. Finally, some CNS species are associated with persistent IMI suggesting that some species (e.g., *Staph. chromogenes* and *Staph. simulans*) are better host-adapted, whereas others may have an environmental reservoir.

Key words: coagulase-negative staphylococcus, mastitis, bovine

INTRODUCTION

The mastitis prevention program put forth by the National Mastitis Council has resulted in improved control of contagious mastitis pathogens on well-managed dairy herds (National Mastitis Council, 2009). As of the implementation of this program, CNS have become the most prevalent group of bacteria found in bovine milk samples in some areas of the world (Pitkälä et al., 2004; Tenhagen et al., 2006; Sampimon et al., 2009). This finding has raised concerns about the overall importance of CNS in milk quality and mastitis.

Currently, the role of the different CNS species in bovine mastitis is not completely understood. Coagulase-negative staphylococci have been cultured from the milk of cows with and without an elevated milk SCC (Thorberg et al., 2009), from the apex of the teat, from other body sites on the cow, and from the environment (Taponen et al., 2008; Piessens et al., 2011). As a group, CNS have been shown to induce only a mild inflammatory reaction in infected quarters, and CNS IMI will most often remain subclinical (Schukken et al., 2009). Some authors, however, consider CNS to be true mastitis pathogens, as they have also been associated with chronic infections (Gillespie et al., 2009) and may carry virulence factors important in mastitis

Received October 30, 2013.
Accepted April 30, 2014.
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pathogenesis (Zhang and Maddox, 2000). In contrast, other studies have found higher milk production in cows with CNS IMI (Compton et al., 2007; Schukken et al., 2009). Conflicting results as to the importance of CNS as mastitis pathogens is likely due to the failure to acknowledge variations within and between these species.

Unfortunately, the number of studies that have identified CNS isolates to the species level is somewhat limited. Also, comparison of the results between studies is difficult because, until recently, many studies used various phenotypic speciation techniques. Phenotypic speciation methods are poorly adapted for identifying CNS isolates of bovine origin (Zadoks and Watts, 2009). Genotypic speciation methodologies have recently been validated for samples of bovine origin. These methods are increasingly being used in research to differentiate CNS isolates because of higher reproducibility and typeability (Mellmann et al., 2006).

Using molecular techniques that allow accurate speciation and fingerprinting of the CNS species, recent research has found variations among species, including effects on SCC, persistence of infection, and epidemiological behavior. For example, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, and *Staphylococcus simulans* have been associated with persistent infections (Piessens et al., 2011; Supré et al., 2011). One study found the same amplified fragment-length polymorphism type from the same mammary quarter for up to 12 repeated monthly samples (Piessens et al., 2011). Other researchers have shown *Staph. chromogenes*, *Staph. simulans*, and *Staph. xylosus* to have a substantial effect on milk SCC, effects comparable to that of *Staphylococcus aureus* (Supré et al., 2011). Diversity has also been found with regards to potential reservoirs of infection. A high level of clonality among *Staph. chromogenes* and *Staph. epidermidis* isolates was noted in one report, suggesting a more host-adapted nature of these species (Piessens et al., 2012). That same study found many different genotypes of *Staph. haemolyticus* and *Staph. simulans* in the environment and cow milk, suggesting an environmental origin of these species.

To the best of our knowledge, no North American studies have been published that used genotypic speciation of CNS isolates and correlated these data with inflammation or persistence of IMI in the mammary gland. One previous North American study found that, within a herd, an average of 15% of cows were infected with CNS, and these cows had a higher milk production than culture-negative cows (Schukken et al., 2009). A more recent publication that evaluated CNS IMI during lactation on 90 Canadian dairy herds found a prevalence of 43% (Dufour et al., 2012). Although these studies screened large numbers of herds, they did not speciate CNS isolates, and therefore were unable to associate any production parameters with specific species of CNS. Another study conducted in the United States found *Staph. chromogenes* to be the most prevalent species of CNS (48%), followed by *Staphylococcus hyicus* (26%), and *Staph. epidermidis* (10%), although that study used a phenotypic speciation method (Gillespie et al., 2009).

The aim of the present study was to evaluate the association between IMI with individual CNS species, milk SCC, and persistence of IMI. To examine these associations in a large population of cattle, CNS isolates from the Canadian Bovine Mastitis Research Network (**CBMRN**; Saint-Hyacinthe, Quebec, Canada) culture collection were obtained for genotypic speciation.

MATERIALS AND METHODS

Sample Selection

A convenience sample of CNS isolates was obtained from the CBMRN culture collection for speciation. This collection of mastitis pathogen isolates originated from a 2-yr study conducted on a cohort of 91 Canadian dairy farms in the years 2007 and 2008, as described by Reyher et al. (2011). During that study, mammary quarter milk samples were collected at regular intervals from cows on the participating farms. Briefly, during 4 different sampling periods between January 2007 and December 2008, 15 apparently normal milking cows were selected in each herd. Mammary quarters of these cows were sampled 3 times at 3 weekly intervals during winter 2007, winter 2008, and summer 2008. Additionally, mammary quarters were sampled once weekly for 7 wk during summer 2007. Furthermore, 15 cows were selected in each herd in 2007 and again in 2008 to follow IMI over the dry period. Mammary quarters of these cows were sampled twice before drying off (at 4 to 2 wk before drying off and between 2 wk and drying-off) and twice after calving (within 24 h of calving and from 1 to 2 wk after calving). As cows were randomly selected, and given the relatively small number of milking cows in these herds (mean = 85 cows/herd; range = 32–326 cows/herd), many cows were selected in more than one series, which resulted in some mammary quarters being sampled up to 23 times over the period of the study. Bacterial isolates cultured from various time points within the dairy production cycle were preserved and available for further characterization. For inclusion of isolates in the CBMRN culture collection, the mammary quarters had to yield growth of at least 10 cfu of morphologically similar CNS from a 10-μL inoculum of milk (i.e., ≥1,000 cfu/mL). This diagnostic criteria was based on the National Mastitis Council guidelines