BOVINE MASTITIS CAUSED BY COAGULASE-NEGATIVE STAPHYLOCOCCI MAY PERSIST

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SUMMARY

Persistence of coagulase-negative staphylococci in intramammary infection during lactation was studied. Milk samples from 328 udder quarters of 82 dairy cows were collected every 4 weeks until the end of lactation. CNS isolates were analyzed with API Staph ID 32 test and genotyped using amplified fragment length polymorphism analysis. In total, 63 CNS infections were detected during lactation. Twenty-nine infections persisted and 34 were transient. Most of the persistent infections lasted until the end of lactation. The SCC was clearly elevated during CNS infection. *Staphylococcus chromogenes* and *Staphylococcus simulans* were the CNS species isolated most often.

Keywords: mastitis, bovine, persistent, coagulase-negative staphylococci

INTRODUCTION

Coagulase-negative staphylococci (**CNS**) in dairy production are often considered pathogens of minor importance, especially in contrast to *Staphylococcus aureus*, streptococci, and coliforms, which may cause severe mastitis. In many countries, CNS-s are the predominant pathogens associated with mastitis (Waage et al., 1999; Macovec and Ruegg, 2003; Nevala et al., 2004) and isolated in the prevalence studies (Wilson et al., 1997; Pitkälä et al., 2004; Tenhagen et al., 2006). For reasons not yet known, CNS infection is especially common in heifers (Honkanen-Buzalski et al., 1994; Aarestrup and Jensen, 1997). CNS mastitis usually remains subclinical or mildly clinical (Taponen et al., 2006), but affects the milk quality by increasing the somatic cell count (**SCC**) (Djabri, 2002), and may slightly decrease milk production (Timms and Schultz, 1987; Gröhn et al., 2004; De Vliegher et al., 2005). Although CNS mastitis is commonly expected to cure spontaneously, there is some evidence that CNS infection may persist in the udder for long times or even for the entire lactation (Aarestrup and Jensen, 1997; Laevens et al., 1997a; Chaffer et al., 1999; Taponen et al., 2006).

Our aim was to investigate the persistence of CNS in the udder of lactating cows over the entire lactation period using consecutive sampling and pheno- and genotyping of the isolates. The influence of CNS infection on the milk SCC was also examined.

MATERIALS AND METHODS

The study was conducted in the research dairy herd of the University of Helsinki. A total of 328 udder quarters of 82 dairy cows (30 primiparous, 52 multiparous) were sampled 2 weeks before calving, at calving, and every 4 weeks thereafter until the end of lactation or until the cow left the herd. CNS isolated from the milk samples were analyzed with API Staph ID 32 test and genotyped using amplified fragment length polymorphism (**AFLP**) analysis. AFLP patterns were used both for similarity analysis between CNS isolates and for species identification. For the latter, AFLP patterns of CNS isolates and 48 staphylococcal type strains were used as operational taxonomic units in numerical analysis. In addition, the somatic cell count (**SCC**) of the milk samples was measured during lactation. CNS infection was determined as persistent if CNS growth was detected in at least three consecutive or almost consecutive samplings (one bacterially negative sample was accepted between two samples with growth of the same CNS strain), and the isolates from these samplings possessed identical AFLP patterns.

RESULTS

CNS infection was detected during lactation in 63 (19.2%) of all 328 quarters. Thirty of these 63 quarters were in 22 primiparous cows and 33 in 25 multiparous cows. Thus, the incidence of CNS infection during lactation was 25.0% for quarters in first lactation and 15.9% for quarters in later lactations. All infections were associated with subclinical or mildly clinical mastitis. Before parturition, at parturition, or both in total 57 quarters were infected with CNS (37.5% of first lactation quarters and 5.8% of subsequent lactation quarters). In 28 of these 57 quarters, CNS infection was detected again during the lactation. Thirty-two quarters were infected at the beginning of lactation and 33 quarters for the first time later during lactation. In cows in first lactation 74.2% of quarters and in cows in later lactations 20.0% of quarters were infected at the beginning of lactation.

CNS infection persisted in 29 quarters (in 13.3% of quarters in first lactation and 6.3% of quarters in later lactations) and was transient in 34 quarters (11.7% of quarters in first lactation and 9.6% of quarters in later lactations). Most persistent infections lasted from the detection of the infection to the end of the lactation or culling of the cow. In 14 quarters with transient infection, the causative strain was isolated twice and in 20 quarters only once.

The mean of geometric means of SCC of quarters with persistent CNS infection was during the infection over 600 000 cells/mL, which is clearly higher than the mean of geometric means of SCC of quarters with no bacterial growth throughout the lactation, which was about 60 000 cells/mL.

According to both API Staph ID 32 test and AFLP analysis, the predominant CNS species both in transient and persistent infections was *S. chromogenes*, followed by *S. simulans*. The API test was unable to identify almost one third of the isolates with an acceptable identification result set at 90% of probability. The agreement in species identification of API and AFLP analyses was 70%.

DISCUSSION

About half of the CNS infections persisted for long periods during the lactation. This result supports earlier evidence, which shows that CNS cause chronic mastitis, and at least some of the CNS infections persist (Aarestrup and Jensen, 1997; Laevens et al., 1997a; Chaffer et al., 1999; Taponen et al., 2006). Most of the quarters with persistent infections remained infected from the detection of infection until the end of lactation. Based on the AFLP patterns, one clone was often isolated from the quarter before and after calving, but about half of the quarters became infected for the first time later during lactation. Multiparous cows were in general infected with CNS in later lactation, whereas primiparous cows usually were infected already in the beginning of lactation. The same has also been shown by Gröhn et al. (2004). In 41% of the transient infections, the same strain was detected twice, the infection thus lasting at least one month. Approximately 50% of the quarters in which a CNS infection was detected before or at calving were cured spontaneously during lactogenesis.

Differences in persistence between CNS species may exist, but in our data with limited number of infected quarters, such a difference between the species was not detected. The same CNS species and isolates with similar AFLP patterns were found in both persistent and transient infections. This suggests that host-microbe interaction plays a key role in the genesis of infection. Heifers and primiparous cows were much more susceptible to CNS mastitis than were multiparous cows. Although this is commonly known, the reason for this phenomenon still remains unknown.

Quality requirements for raw milk are high and the price of bulk tank milk is often connected with the somatic cell count (**SCC**) of the milk. In Finland, the requirement for best bulk milk price is SCC <250 000 cells/mL and bacterial count <50 000/mL. The dairy producers pay much attention to keeping the SCC low, and any bacteria persisting in the udder and increasing the SCC are in this respect harmful. Compared with infections caused by other common Gram-positive mastitis pathogens, such as *S. aureus* and streptococci, the SCC in quarters infected with CNS is rather low. It is, however, about 10-fold higher than the SCC of healthy quarters, which typically remains under 50 000 cells/mL (Barkema et al., 1999). We found the SCC of a healthy quarter to remain between 10 000 and 40 000 cells/mL in both first and later lactations, whereas in CNS infections the SCC is clearly elevated. The SCC in quarters with persistent CNS infection considerably varied from sampling to sampling, and so the geometric means of quarters infected with CNS. Compared with the study of Djabri et al. (2002), the mean SCC of quarters with CNS infection was here somewhat higher than the average SCC in that meta-analysis. Even a transient CNS infection caused a temporary increase in SCC, which is consistent with the report of Laevens et al. (1997b). CNS infection induces an immunological reaction in the udder and should not be considered merely teat canal colonization or normal situation for the udder.

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