Streptococcus parasanguinis: New Pathogen Associated with Asymptomatic Mastitis in Sheep

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We describe two unusual cases in sheep of subclinical mastitis caused by *Streptococcus parasanguinis*. This bacterium has been associated with the development of experimental endocarditis; its presence at relatively high concentrations in apparently healthy sheep milk may pose a health risk in persons with predisposing heart lesions.

Ovine mastitis represents a major sanitation and economic problem for both milking sheep farmers and the sheep-milk cheese industry because it reduces milk production and quality. Many microorganisms produce asymptomatic, or subclinical, mastitis in milking sheep. Coagulase-negative staphylococci, particularly *Staphylococcus epidermidis*, are most prevalent (1,2,3), although other bacterial groups have recently emerged as clinically important (4). Streptococci are also responsible for a significant proportion of cases; however, while a large number of species of staphylococci cause subclinical mastitis, the number of streptococci species found to do so is limited, usually to *Streptococcus agalactiae*, *S. uberis,* and *S. dysgalactiae* (3,5).

We report two unusual cases of subclinical mastitis in sheep involving *S. parasanguinis* (6,7). This atypical viridans *Streptococcus* bacterium has been isolated from humans (throat, blood, and urine) (7) but has not been associated with mastitis. The viridans group streptococci are the most common pathogens associated with native valve endocarditis, a disease of increasing medical importance in industrialized countries (8). In patients with this disease, eating and teeth brushing cause low-grade bacteremia, which allows circulating bacteria to adhere to the damaged endocardium (9). Although *S. parasanguinis* has not yet been established as a human pathogen, it has been clearly associated with the development of experimental endocarditis (10). Therefore, the excretion of *S. parasanguinis* at relatively high concentrations in milk from apparently healthy sheep is of concern. Indeed, its presence in certain dairy products, such as nonpasteurized handmade cheeses, may pose a health risk in persons with predisposing heart lesions.

The two isolates in our study were recovered in 1997 from two sheep of an Assaf flock during a bacteriologic survey for determining the prevalence of subclinical mastitis in Madrid (central region of Spain). Affected sheep did not show signs of clinical mastitis or milk abnormalities but had California Mastitis Test (CMT) scores of 2+. CMT estimates the degree of inflammation of the mammary gland by detecting increased numbers of leukocytes in milk (11). The bacteriologic counts of the milk samples were 1×10^4 CFU/ml in one of the sheep and 1.7×10^3 CFU/ml in the other. Mammary glands with these characteristics (no clinical abnormalities, apparently normal milk secretion, positive for CMT, and bacteriologically positive) are routinely considered to have subclinical mastitis (1,12). Milk samples were collected and analyzed as described previously (13). After 48 hours of incubation at 37°C under aerobic conditions on Columbia blood agar, pure cultures of α-hemolytic colonies were recovered from both milk samples.

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The isolates are clinically important because both sheep fit the criteria for subclinical mastitis and the isolates were recovered in pure culture.

Both strains were catalase-negative gram-positive cocci, and biochemical identification was attempted with the commercial rapid ID 32 Strep system (Bio-Mérieux S.A.). Both isolates had an identical biochemical profile, which did not correlate with any of the species identified with the ID 32 multisubstrate strip. Sequencing of 16S rRNA genes provided a definitive identification of the streptococci isolates as *S. parasanguinis*. This molecular technique is a powerful method for determining the identity of bacteria that cannot be properly identified by conventional physiologic or biochemical approaches and has proven powerful for describing new bacterial pathogens causing mastitis (13,14). The 16S rRNA gene of each isolate was amplified by polymerase chain reaction (PCR) and was sequenced to determine genotypic identity (13,14). The determined sequences consisted of more than 1,400 nucleotides (representing more than 95% of the total 16S rRNA gene) and were compared with the sequences of other streptococcal species available in the European Molecular Biology Laboratory Database Library. The 16S rRNA gene sequence analysis showed both isolates to be genotypically identical and to display 99.7% sequence similarity with the strain 85-81 of *S. parasanguinis*, which on the basis of DNA-DNA hybridization studies was shown to be a true member of this species (7).

The two *S. parasanguinis* isolates represented 7% of our total streptococci isolated from cases of subclinical mastitis. According to these data, only sporadic cases of mammary gland infections due to *S. parasanguinis* would be expected. However, because many streptococcal isolates are usually identified only to the genus level (2,3,11,15), the diversity of species involved and the incidence of infection due to particular streptococcal species are difficult to know. The same difficulty is true with corynebacteria (4).

The recognition of *S. parasanguinis* as a new animal pathogen causing subclinical mastitis in sheep and the recent recognition of new *Corynebacterium* species associated with this disease (13,14) suggest the need for epidemiologic surveys to determine the range of species diversity, other than coagulase-negative staphylococci, involved in subclinical sheep mastitis. According to recent results, this range appears higher than previously considered. Particular attention should be paid to species potentially pathogenic for humans.

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