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Meningoencephalitis Associated with *Globicatella sanguinis* Infection in Lambs

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Thirty lambs displayed symptoms of meningoencephalitis. An unusual gram-positive coccus was isolated in pure culture from the blood and brain samples from one of the affected animals, and phenotypic and phylogenetic characterization showed this to be *Globicatella sanguinis*. This is the first report of the isolation of *G. sanguinis* in pure culture from an animal infection.

Globicatella sanguinis was described in 1992 as a new genus and species of catalase-negative, facultatively anaerobic, gram-positive cocci (3). The organism has been recovered from a variety of human clinical specimens, including blood of bacteremic patients, urine of patients with urinary tract infections, cerebrospinal fluid of a patient with meningitis, and wounds (3, 10). Meningitis and meningoencephalitis are two of the most frequent pathologies affecting the central nervous system in domestic animals. A broad range of microorganisms are associated with these conditions in small ruminants (2), although *Listeria monocytogenes* is the pathogen most frequently implicated (13–15). In this paper, we describe an unusual outbreak of meningoencephalitis in lambs produced by *Globicatella sanguinis*. To our knowledge, this is the first report of the association of *G. sanguinis* with animal disease.

Case report. Thirty 8-month-old lambs out of a total of 156 lambs within a flock located in the province of Toledo, central region of Spain, developed neurological disorders characterized by depression, lack of appetite, ataxia, nystagmus, and pressing the head against the floor. These clinical symptoms are similar to those produced by *L. monocytogenes*, and this bacterium was initially considered to be the etiological agent, based on the presumptive clinical diagnosis. Twelve of the affected lambs died between 2 and 5 days after the onset of the symptoms (case fatality rate, 40%). One diseased lamb was sacrificed and necropsied. Macroscopic lesions were confined to the central nervous system, with congestion and petechiae in the meninges. Histologic examination revealed a suppurative meningoencephalitis. Gram-positive cocci were observed inside the meningeal vessels, suggesting a hematogenous dissemination as the likely route of entry of these organisms into the central nervous system. Culture of the blood and brain yielded a gram-positive coccus, which was subsequently identified as *G. sanguinis*.

Microbiology and identification. Samples of blood (obtained prior to the sacrifice) and brain of the necropsied lamb were taken for bacteriological examination. Blood was cultured according to conventional protocols (9) with Hemoline performance Diphasique medium (bioMérieux España, s.a.). The brain sample was inoculated on Columbia blood agar (bioMérieux España, s.a.), which was incubated under aerobic and anaerobic conditions at 37°C. Pure cultures of α-hemolytic, facultatively anaerobic, gram-positive, catalase-negative, coccus-shaped organisms were isolated from both samples. Biochemical identification was attempted with the commercial rapid ID 32 Strep (bioMérieux España, s.a.). Both isolates showed identical biochemical profiles, which did not match any of the species identified with this commercial system. Molecular genetic identification of the clinical isolates was attempted by sequencing of the 16S rRNA gene of the brain isolate, as described previously (6). This molecular technique has been shown to be extremely useful for the identification of unusual animal pathogens (5, 6, 8, 11). The 16S rRNA gene sequence determined displayed 99.5% similarity to that of the type strain of *G. sanguinis* (CCUG 32999^T). The brain isolate was subjected to further phenotypic characterization and compared directly with the type strain of *G. sanguinis* by using the commercial API 20 Strep and rapid ID 32 Strep systems (bioMérieux España, s.a.). Growth in 6.5% NaCl, growth at 45 and 10°C, and susceptibility to vancomycin were also determined (10). *G. sanguinis* CCUG 32999^T and the clinical strain produced acid but not gas from glucose. They grew in 6.5% NaCl, but failed to grow at 10 and 45°C, and were susceptible to vancomycin. Both strains also displayed biochemical patterns identical to those of the commercial identification systems. The API rapid ID 32 Strep profile 62376273750 did not match any species identified by this system, whereas the API 20 Strep profile 6116577 corresponded to an unacceptable identification of *Aerococcus viridans* 1. The phenotypic findings overall are consistent with those described for *G. sanguinis*, thereby reinforcing the 16S rRNA identification. However, contrary to earlier data (3), the type strain did not produce acid from sorbitol. The acidification of sorbitol is, however, variable for *G. sanguinis* (7). The brain isolate has been deposited in the Spanish Type Culture Collection as *G. sanguinis* CECT 5299.

Discussion. The isolation of *G. sanguinis* in pure culture both from blood and brain is strongly indicative of the clinical significance of this isolate. Although the disease was bacteriologically confirmed only in one lamb sent to the laboratory, and no bacteriological analysis of the other affected lambs was performed, the fact that all animals had identical clinical signs is strongly indicative that *G. sanguinis* was also responsible for the disease in the other lambs. This report indicates that *G.*

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sanguinis should be included in the list of possible etiological agents of disease showing neurological signs in small ruminants. It is pertinent to note that *G. sanguinis* is one of a plethora of gram-positive catalase-negative coccus-shaped taxa described from human and/or animal clinical sources in the last decade (e.g., *Helcococcus* [4, 6, 12], *Facklamia* [7], and *Dolosigranulum* [1]). Although the identification of *G. sanguinis* and other newly described organisms can be achieved by phenotypic tests, difficulties can often occur, and the use of molecular genetic tools such as 16S rRNA gene sequencing should be encouraged for the identification of such problematic veterinary organisms. This would greatly improve our knowledge of the host distribution, range of clinical conditions, and significance of these unusual gram-positive catalase-negative taxa.

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