Unusual Outbreak of Clinical Mastitis in Dairy Sheep Caused by *Streptococcus equi* subsp. *zooepidemicus*

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Received 4 June 2001/Returned for modification 23 September 2001/Accepted 4 November 2001

This work describes an outbreak of clinical mastitis affecting 13 of 58 lactating ewes due to *Streptococcus equi* subsp. *zooepidemicus*. *S. equi* subsp. *zooepidemicus* was isolated in pure culture from all milk samples. All the clinical isolates had identical biochemical profiles and antimicrobial susceptibility patterns and also exhibited indistinguishable macrorestriction patterns by pulsed-field gel electrophoresis, indicating that all cases of mastitis were produced by a single strain.

Streptococci are probably the second group of microorganisms in importance, after staphylococci, responsible for mastitis in sheep (5). Although *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae* are the species more frequently identified, other species of streptococci, such as *Streptococcus parasanguinis*, have been implicated in mammary gland infections (12). In this paper we describe an unusual outbreak of mastitis in sheep produced by *Streptococcus equi* subsp. *zooepidemicus*. This Lancefield group C streptococcus is part of the normal flora of the respiratory and urogenital tracts of equines, and it could be considered the most prevalent agent responsible for equine mastitis (17). It has also been implicated in sporadic cases of cattle and goat mastitis (9, 15, 20). However, to our knowledge, this is the first report of the association of *S. equi* subsp. *zooepidemicus* with sheep mastitis.

An outbreak of clinical mastitis due to *S. equi* subsp. *zooepidemicus* infection was detected in a flock of 170 crossbred Awassi-Rubia de El Molar sheep. The animals were kept under extensive conditions, with at least 8 h per day of grazing in natural pastures during the whole year, and their diet was supplemented with alfalfa hay and commercial concentrates. The sheep had no contact with other animal species except for a male donkey used by the shepherd to transport his equipment. The ewes were housed in a barn during the night, and the hygiene conditions of the barn and the welfare of the animals were considered up to standard. The lambs were usually weaned at the age of 21 days. The ewes were hand milked twice daily by the same shepherd. The hygiene conditions for milking were not adequate. The shepherd never used gloves for milking and did not wash his hands between animals. No specific control measures against mastitis were taken. The outbreak involved 13 of 58 ewes in lactation (morbidity rate, 22%). Cases of mastitis were detected about 30 days after weaning, and they were clustered in 2 weeks after the first case was detected. No more cases of mastitis were further recorded.

Mastitis was always unilateral. None of the affected ewes exhibited acute inflammation of the udder, and no systemic signs of illness were observed either. The milk secretion became watery, containing small flecks of pus. The average daily milk production of healthy ewes, ranging between 500 and 900 ml, decreased drastically in the affected mammary glands, which became dry 5 days after the onset of symptoms. The animals were treated intramuscularly with oxytetracycline (Terramycin-La; Pfizer) (two doses of 600 mg with a 3-day interval in between) without success. No ewe died, but none recovered milk secretion in the affected gland. The affected animals were prematurely dried off and sent to the slaughterhouse. Similar clinical courses and morbidity rates have been reported for cattle and goat mastitis caused by *S. equi* subsp. *zooepidemicus* (9, 20).

Milk from the affected mammary glands was collected in sterile vials after disinfecting the teat end and kept under refrigeration during transportation to the laboratory for microbiological analysis. Milk samples were surface plated on Columbia blood agar (bio-Mérieux España, s.a.) and incubated aerobically for 48 h at 37°C. Pure cultures of hemolytic facultative anaerobic, gram-positive, catalase-negative cocci were isolated from all milk samples. The Lancefield group was determined by using the SlideX Strepto-kit (bio-Mérieux España, s.a.). The CAMP test with *Staphylococcus aureus* was also performed according to the recommendations of Quinn et al. (19). Biochemical identification was achieved using the commercial systems Rapid ID 32 Strep version 2.0 and API 20 Strep version 6.0 (bio-Mérieux España, s.a.). All the clinical isolates (SH1 to SH13) were of Lancefield group C, gave negative CAMP reactions, had identical biochemical profiles by both systems, and were identified as *S. equi* subsp. *zooepidemicus*. Quantitative detection of *S. equi* subsp. *zooepidemicus* was determined (14) in three samples with counts ranging from $5.7 \times 10^3$ to $1.1 \times 10^4$ CFU/ml, suggesting a high excretion rate of this microorganism in milk. Susceptibility to antimicrobial agents was determined by the disk diffusion method on Mueller-Hinton blood agar (Bio-Mérieux España, s.a.) following the National Committee for Clinical Laboratory Standards guidelines (18). All the clinical isolates displayed identical zone...
The presence of a common source of infection.

Infections by this microorganism in animal species other than mares have been associated with hand milking, and the contact with horses was suspected to be the origin of the infection (7, 20, 22). In this outbreak a donkey present on the farm shared pastures with the ewes during grazing and was housed in their sheepfold. The donkey was apparently healthy, with no recent history of respiratory illness, and *S. equi* subsp. *zooepidemicus* is part of the normal flora of equine. Thus, its daily contact with the ewes may have allowed an opportunity for exposure to infection. Additionally, the fact that milking was done without proper hygiene conditions is a risk factor that may have contributed to the transmission of *S. equi* subsp. *zooepidemicus* from the donkey to the mammary glands of ewes through the hands of the shepherd during milking, as well as to the spread of the infection among ewes. However, the implication of the donkey as the source of the infection could not be experimentally demonstrated because no specific epidemiological investigation of this microorganism (3). All *S. equi* subsp. *zooepidemicus* isolates were molecularly characterized by PFGE according to the specifications of Vela et al. (23). The restriction enzymes *Apa*I (Promega Co.) and *Sma*I (MBI Fermentas) were used according to the manufacturer’s recommendations. Both enzymes have been successfully applied in the molecular typing of streptococci (4, 10, 21). All the clinical isolates displayed indistinguishable banding patterns by PFGE with *Sma*I (data not shown) and *Apa*I (Fig. 1) restriction enzymes, indicating that all the clinical cases of mastitis were produced by a single strain. The PFGE results suggested the existence of a common source of infection.

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Our overall frequency of isolation of *S. equi* subsp. *zooepidemicus* from clinical mastitis in small ruminants is about 4.7% of the isolates (A. Las Heras, unpublished data). Although the number of cases of mastitis due to this microorganism is lower than the number of cases produced by other bacterial species (5), the high morbidity rates, together with the decrease in milk production and the premature drying off of the animals, make mammary gland infections by *S. equi* subsp. *zooepidemicus* a serious sanitary and economic problem. *S. equi* subsp. *zooepidemicus* is also responsible for various sporadic human infections such as bacteremia, meningitis, septicemia, or endophthalmitis (1, 16), as well as for outbreaks associated with the consumption of nonpasteurized milk or milk products (3, 8, 11). Thus, the excretion of this pathogen in milk represents for consumers of raw sheep milk products a health risk that should not be underestimated.

This work has been partially supported by the AGF98-0829 project in response to the Spanish Ministry of Education and Culture and by the Cooperativa Castellana de Ganaderos (Campo Real, Madrid). A. Las Heras and E. Fernández are recipients of a predoctoral grant assigned by the Spanish Ministry of Education and Culture.

REFERENCES