J. Dairy Sci. 92:943–951
doi:10.3168/jds.2008-1548

Epidemiological investigation of *Streptococcus equi* subspecies *zooepidemicus* involved in clinical mastitis in dairy goats

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ABSTRACT

An outbreak of clinical mastitis was observed in dairy goats due to the zoonotic pathogen *Streptococcus equi* ssp. *zooepidemicus*. Affected goats were culled to prevent transmission of infection to other animals or humans. The objective of the study was to determine whether horses on the same farm were the source of the pathogen. *Streptococcus equi* ssp. *zooepidemicus* was obtained from milk of 10% of goats in the herd and from feces of 3 of 7 healthy horses that shared pasture and housing with the goats. Isolates of caprine and equine origin had identical biochemical profiles, including the ability to ferment sorbitol and lactose, which distinguishes *S. equi* ssp. *zooepidemicus* from *S. equi* ssp. *equi*. Sequencing of the 16S–23S intergenic spacer region and results from *sodA*-*seeI* multiplex PCR supported identification of isolates as *S. equi* ssp. *zooepidemicus*. Based on random amplified polymorphic DNA typing and *rpoB* and *sodA* sequencing, caprine isolates were indistinguishable from each other, but distinct from equine isolates. Further analysis of equine fecal samples showed that multiple strains of *S. equi* ssp. *zooepidemicus* can be present in a single sample or in sequential samples obtained from a single horse. Failure to detect the mastitis-causing strain in equine feces may indicate that horses were not the source of the mastitis outbreak in goats. Alternatively, the outbreak may be due to presence of multiple *S. equi* ssp. *zooepidemicus* strains in equine feces and a failure to detect all strains when analyzing a limited number of isolates per sample.

Key words: *Streptococcus equi* ssp. *zooepidemicus*, goat, horse, mastitis

INTRODUCTION

Streptococcus equi ssp. *equi* (**SEE**) and *Streptococcus equi* ssp. *zooepidemicus* (**SEZ**) are β-hemolytic group C *Streptococcus* species that cause disease in animals and humans. Phylogenetically, SEZ is regarded as the ancestral species of the closely related clonal subspecies SEE (Chanter et al., 1997; Harrington et al., 2002; Timoney, 2004). Identification and differentiation of subspecies was traditionally based on biochemical reactions such as hydrolysis of esculin, fermentation of sorbitol, and fermentation of trehalose (Harrington et al., 2002). Recent developments in nucleic acid technology resulted in new methods for differentiation of the 2 subspecies, such as PCR-mediated identification of species-specific segments of the 16S–23S rDNA intergenic spacer regions (**ISR**; Chanter et al., 1997; Hassan et al., 2003). *Streptococcus equi* ssp. *zooepidemicus* is commonly found colonizing the mucous membranes of healthy equids. It is associated with respiratory tract infections in foals and with uterine infections in mares (Timoney, 2004). Moreover, SEZ is a possible cause of mastitis in dairy ruminants (Sharp et al., 1995; Las Heras et al., 2002). The IMI caused by SEZ were named pseudo-agalactia because the clinical signs were similar to those observed in chronic contagious agalactia due to *Mycoplasma* spp. Previous studies described cases of clinical mastitis in dairy ruminants and hypothesized the role of equids as the origin of infections (Sharp et al., 1995; Bezek, 1998; Las Heras et al., 2002). None of the studies cited employed molecular typing techniques to compare SEZ strains involved in mastitis outbreaks to isolates from other host species. Unlike SEE, SEZ is found in human infections. Human infections are rare and the clinical presentations include pharyngitis, septicemia, meningitis, purulent arthritis, and endocarditis (Barnham et al., 1983; Balter et al., 2000). The source of human infection was often traced back to contact with domestic animals, especially horses, or ingestion of unpasteurized milk or milk products (Edwards et al., 1988; Bordes-Benítez et al., 2006; Kuusi et al., 2006). For example, this organism was associated with a large

Received July 14, 2008.
Accepted October 20, 2008.
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outbreak of nephritis in Brazil that was attributed to consumption of unpasteurized cheese (Balter et al., 2000).

An outbreak of SEZ mastitis is described in a goat herd that produced milk for the manufacture of raw milk cheese. Based on molecular typing, horses that were present on the same farm as the goats were investigated as the probable source of infection. Methodological aspects of this case are discussed, and suggestions for improvement of methodology in future outbreak analysis are made.

MATERIALS AND METHODS

Case History

A herd of goats on a small-scale commercial dairy farm in northern Italy (Varese, Lombardy) was monitored monthly throughout lactation (i.e., from March to September) for milk quality and production traits. Technicians of the Provincial Breeding Association of Varese (Lombardy) collected milk samples for measurement of SCC, fat and protein content, and milk yield. In addition, milk samples were collected aseptically from each udder half for routine bacteriological analysis. The herd grazed on natural pastures for at least 8 h daily. Animals were housed in a barn during the night. The ration was supplemented with concentrates, and water was available at all times. The farm practiced seasonal milking and the does kidded between January and February. After weaning, all goats were hand-milked twice daily inside the barn by the same milker without teat preparation or cleaning. Milk was taken in a plastic box for daily milk yield measurement, collected in a bucket, and then stored in a refrigerated bulk tank. Milk was used to manufacture raw cheeses. Teat disinfection and application of antimicrobials during the dry period were not practiced. The farm was free of brucellosis and mycoplasmosis. The goats had no contact with other dairy ruminants, but they shared pastures and a barn with 7 horses.

At the end of May, the farmer observed the appearance of clinical signs of mastitis in 2 of 22 lactating goats. Mammary glands appeared swollen, firm, and painful at palpation, and the milk contained small clots. Daily milk production decreased in the affected mammary glands, which dried up 2 to 3 d after onset of symptoms. Affected goats showed systemic signs of illness (fever, anorexia, depression, and decreased rumen motility). Infected animals were segregated from the herd as a preventive measure to avoid goat-to-goat transmission. No goats died, but the affected animals were prematurely slaughtered as soon as the bacteriological diagnosis was known because of the contagious and zoonotic nature of the pathogen. No further cases of clinical mastitis were observed in the herd during the remainder of the lactation.

Milk Sample Processing

Milk samples were collected aseptically from individual half-udders of all goats. Teat ends were cleaned and disinfected with chlorhexidine before sampling. The first streams of foremilk were discarded, and 10 mL of milk was collected into sterile vials. Samples were kept under refrigeration until bacteriological analysis was performed using standard methods (National Mastitis Council, 1999). Briefly, 10 μL of each milk sample was spread on blood agar plates (5% defibrinated sheep blood, Microbiol Diagnostici, Cagliari, Italy). Plates were incubated aerobically at 37°C and examined after 24 h. β-Hemolytic colonies were provisionally identified as streptococci based on Gram stain, morphology, and catalase reaction. The Christie-Atkins-Munch-Petersen (CAMP) test was performed using conventional procedures (National Mastitis Council, 1999). The isolates were further characterized biochemically using commercial methods (API 20 Strep, BioMérieux, Rome, Italy) following the instructions of the manufacturer.

Fecal and Nasal Sample Processing

Fecal samples were collected using single-use plastic gloves from the rectum of 7 horses that shared the same pastures and barn as the goats. Samples were kept at 4°C and processed within 24 h of collection. For detection of presumptive *Streptococcus* spp., 1 g of fecal matter was suspended in 9 mL of sterile buffered peptone saline solution (Merck KGaA, Darmstadt, Germany) and 10 μL of the suspension was spread on blood agar plates.

A single nasal swab was collected from each horse. A cotton-tipped culture swab (BD Diagnostic Systems, Milan, Italy) was inserted approximately 10 cm into 1 nasal passage and withdrawn with the swab in contact with the nasal mucosa. Swabs were promptly placed in transport medium (Cultiplast with Amies, LP Italiana, Milan, Italy) and kept at 4°C until processing. Swabs were placed in 2 mL of enrichment broth consisting of 10 g/L of tryptone T, 75 g/L of sodium chloride, and 2.5 g/L of yeast extract (Merck KGaA) and incubated aerobically at 37°C for 24 h. Approximately 100 μL of broth was then inoculated onto blood agar plates.

Inoculated blood agar plates were incubated aerobically at 37°C and examined after 24 h. Isolates were subsequently processed as described for milk samples. If a horse tested positive for SEZ in a fecal sample, further fecal samples were collected at subsequent herd