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Relationship among specific bacterial counts and total bacterial and somatic cell counts and factors influencing their variation in ovine bulk tank milk

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ABSTRACT

To analyze the relationship among the counts of different organisms and total bacterial count (BTTBC) and somatic cell count (BTSCC) as determined in dairy laboratories in ovine bulk tank milk, 751 bulk tank milk samples from 205 dairy sheep flocks belonging to Consortium for Ovine Promotion (CPO) were collected between January and December 2011. Four samplings were carried out in each flock, once per season, throughout 1 yr. Variables analyzed were bulk tank counts of thermoduric, psychrotrophic, coliform, and gram-positive catalase-negative cocci (GPCNC) bacterial groups. Thermoduric, psychrotrophic, and coliform species were significantly related to BTTBC, whereas GPCNC were correlated with both BTTBC and BTSCC variables. Highest counts were for psychrotroph and coliform groups, and a moderate to high correlation (r = 0.51) was found between both variables, indicating that poor cleaning practices in the flocks tend to select for less-resistant organisms, such as gram-negative rods. In addition, BTTBC correlated with BTSCC (r = 0.42). Some variation factors for specific bacterial counts, such as breed, season, milking type, dry therapy, and milk yield, were also analyzed. Flock information was collected from flock books, annual audits, and the CPO traceability system. Psychrotrophs and coliforms had elevated counts in winter, whereas GPCNC were higher in summer and in hand-milked flocks. Dry therapy contributed to the reduction in psychrotrophic bacteria; therefore, some strains of mammary pathogens could also be psychrotrophic bacteria. Results of this study would be helpful for troubleshooting milk quality problems and developing premium payment systems in dairy sheep.

Key words: bulk tank milk, dairy sheep, milk quality, milk organisms

INTRODUCTION

In dairy sheep, bulk tank total bacterial count (**BTTBC**) and bulk tank SCC (**BTSCC**) are important but nonspecific tools used by technicians and farmers to evaluate the efficiency of production processes, cleaning and sanitation practices, and flock mammary health, and to predict the keeping quality and shelf life of milk and dairy products. In milk testing and dairy interprofessional laboratories, BTSCC determination is automated by Fossomatic devices (Foss Electric, Hillerød, Denmark), and several researchers have investigated the global accuracy of rapid SCC counters in ovine milk (Gonzalo et al., 2004). Similarly, BTTBC is routinely determined using automated flow cytometry devices (i.e., Bactoscan FC, Foss Electric), and several researchers have explored the global accuracy of Bactoscan and the high correlation between Bactoscan and mesophilic bacterial counts in milk of small ruminants (Tomáska et al., 2006; Sierra et al., 2009).

Bulk tank milk is contaminated by bacteria from different sources such as flora and pathogens present in beds, milking facilities, wash water, milking system, udder, or mastitic milk. Some of these bacteria are resistant to pasteurization or are able to grow at low temperatures, and some indicate fecal contamination or mastitis. Despite this, only the aerobic mesophilic count (i.e., BTTBC) has been the target of various legal limits for ovine milk in the European Union. In this context, other bacterial groups such as thermoduric bacteria, psychrotrophic bacteria, coliforms, and gram-positive catalase-negative cocci (**GPCNC**) would be indicators of interest for milk hygiene, udder health, safety, quality, and marketing (Jayarao et al., 2004) and likely related to both BTTBC and BTSCC. Indeed, high counts of thermoduric species can be caused by unclean equipment or improper sanitizing practices, and these thermoduric bacteria may cause spoilage in pasteurized milk. Coliform count is a practical indicator of milking hygiene and is often correlated with the population of other bacteria in bulk tank milk (Jayarao et al., 2004; Pantoja et al., 2009); coliforms are also im-

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portant mastitis pathogens (Hogan and Smith, 2003). Psychrotrophic microorganisms enter the milk from soiled animals and dirty equipment and are selected by refrigeration of raw milk. Gram-positive catalase-negative cocci include significant groups such as streptococci and enterococci. Mastitis-causing streptococci are important contributors to BTTBC and BTSCC in dairy cattle (Keefe, 1997; Zadoks et al., 2004); thus, control of streptococci is important for improving the microbial and sanitary quality of raw milk. Enterococci are environmental organisms, mostly of fecal origin, that contaminate milking equipment and bulk storage tanks (Gelsomino et al., 2001) and cause mastitis and high SCC in dairy sheep (Marogna et al., 2010). Enterococci are also important for ripening and aroma development of certain traditional cheeses, especially those produced in the Mediterranean region, and they may be used as starter or probiotic cultures (Franz et al., 2003). In addition, some GPCNC are described as emergent opportunistic or nosocomial pathogens for humans (Giraffa, 2002; Franz et al., 2003).

In spite of the importance of the above-mentioned bacterial groups on milk quality, their determination in testing laboratories is expensive and laborious, and analytical automated procedures have not yet been developed. Therefore, the relationship among these bacterial groups and BTTBC and BTSCC is of great interest for monitoring the hygiene quality of bulk tank milk in dairy sheep. Nevertheless, little information is available about the microbial factors influencing variation and covariation of bulk tank milk variables, such as BTTBC and BTSCC, which can be determined on a large scale by automated procedures (Gonzalo et al., 2006), and no known studies have examined the variation of bulk tank bacterial groups (e.g., thermoduric, psychrotrophs, coliforms, or streptococci) and their relationship with BTTBC and BTSCC in dairy sheep. Wider knowledge of BTTBC and BTSCC and their associations with other bulk tank bacterial counts would enable decisions to be made on improving milk quality, farm management practices, and flock udder health.

The goals of this study were (1) to analyze the relationship among bulk tank thermoduric, psychrotrophic, coliform, and GPCNC microbial counts and automated BTTBC and BTSCC, and (2) to identify sources of variation in each bacterial group, such as breed, season, milking system, antibiotic dry therapy, and milk yield in dairy sheep.

MATERIALS AND METHODS

Flocks

Between January and December 2011, a total of 751 bulk tank milk samples were collected from 205 dairy sheep flocks (172 Spanish Assaf and 33 Churra; 182 machine milked and 23 hand milked) belonging to 2 cooperatives of the Consortium for Ovine Promotion (**CPO**) in the Castilla y León region of Spain. Average flock size was about 400 ewes, which is representative of the region; lambing is mostly concentrated in the late fall, winter, and spring, and the characteristic features of these flocks have been previously described (Gonzalo et al., 2005). Briefly, each herd is generally divided into 2 lots, the lambing periods of which alternate every 4 to 6 mo in the herd. Some herds undergo reproductive intensification to achieve 3 lambings every 2 yr, whereas in others, ewes have only 1 lambing per year. Teat washing was not done in any flocks before milking, as this is not a typical practice in dairy sheep.

The information recorded in CPO flocks included the following variation factors: flock, breed, season, milking type (hand or parlor machine milking), annual milk yield, total number of ewes per flock, and antibiotic dry therapy practice in each flock. Dry therapy is administered under veterinary supervision and, when implemented, all animals were treated at the beginning of the dry period (i.e., complete dry therapy). Clinical outbreaks of contagious agalactia were also reported and *Mycoplasma agalactiae* was isolated in bulk tank milk. Annual audits are performed in the CPO flocks and a field database is continuously maintained with above-mentioned information. The CPO veterinary services are centralized and information concerning animal health and antibiotic treatment along with new animals and falls are recorded in the each flock book. Milk yield was recorded from receipt in the CPO traceability system.

Sampling

Throughout the year, 4 samplings were carried out in each flock, once per season. In total, 751 bulk tank samples were analyzed; the 69 missing samples corresponded mostly to flocks that were not in lactation at sampling season and, in a few cases, to nonuseful samples. In each sampling, 2 samples of 50 mL were aseptically collected in sterile containers after milk homogenization according to standards recommended by the American Public Health Association (White et al., 1992). One of the 2 samples was preserved with azidiol (3.3 μL/mL, Panreac Quimica S.A., Castellar del Valles, Barcelona, Spain) according to de Garnica et al. (2011). Sampling was always done immediately before loading the bulk tank milk into the tankers. Milk tanker collection frequency in flocks was 48 h, and milk was kept at <6°C in cooling tanks on the farms in between collections. Milk samples were kept at 4°C until bacteriological analysis, which was carried