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Staphylococcus aureus and *Escherichia coli* prevalence in ovine bulk tank milk

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ABSTRACT

To analyse the effect of bulk tank *Staphylococcus aureus* and *Escherichia coli* presence on total bacterial count (BTTBC) and somatic cell count (BTSCC), and to study the factors influencing their prevalence, a total of 752 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, one per season, throughout one year. Respectively, *Staph. aureus* and *Esch. coli* were present in 26.5% and 17.4% of the samples and in 58.5% and 50.7% of the flocks throughout the year. *Esch. coli* significantly contributed to BTSCC and BTTBC variation, but only a statistical tendency for increased BTSCC was evidenced in the case of *Staph. aureus* positive samples. Thus, BTSCC and BTTBC were useful variables for monitoring, at least partially, the presence of *Esch. coli* in bulk tank milk, whereas BTSCC was more useful in the case of *Staph. aureus*. Some variation factors for specific pathogens, such as season, antibiotic dry therapy, milking type and breed, were also analysed. Season was the most important effect associated with the variation of bulk tank *Staph. aureus* and *Esch. coli* prevalence. *Staph. aureus* had a higher prevalence in winter and spring (i.e., early lactation period), while *Esch. coli* prevalence was higher in autumn and winter, coinciding with a rainy weather. Lower *Staph. aureus* prevalence was for dry-treated flocks pointing out this mastitis control practice in the flocks is related with a reduction of contagious mammary pathogens. Finally, hand milking flocks evidenced a higher prevalence for both pathogens than those with machine milking. As a whole, these results are useful for monitoring these pathogens in bulk tank milk and highlight the need for establishing analytical surveillance programmes in dairy sheep flocks.

1. Introduction

Bulk tank somatic cell count (BTSCC) and total bacterial count (BTTBC) are principal tools used to evaluate udder health and hygiene in dairy cattle (Jayarao et al., 2004; Rysanek et al., 2009) and in dairy sheep and goats (Contreras et al., 2003; Pirisi et al., 2007; Gonzalo et al., 2010). In milk quality-testing or dairy laboratories, both variables are routinely determined using automated flow cytometry devices (i.e., Bactoscan and Fossomatic) (Gonzalo et al., 2004; Tomáska et al., 2006; Sierra et al., 2009).

Contagious or environmental pathogens could provide increased BTSCC and BTTBC (Keefe, 1997; Zadoks et al., 2004; Pantoja et al., 2011), and differential culturing of bulk milk may help farmers to find the cause of high BTSCC or BTTBC. *Staphylococcus aureus* and *Escherichia coli* are two important organisms, which can be isolated from ovine bulk tank milk (de Garnica et al., 2011), although their

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relationship with BTSCC or BTTBC has not been yet studied in dairy sheep. Several studies in dairy cattle showed that a higher frequency of isolation of *Staph. aureus* and *Esch. coli* was significantly related to BTSCC (Hogan and Smith, 2003; Rysanek et al., 2007), and that *Esch. coli* prevalence is a functional indicator of milking hygiene, related to BTTBC (Jayarao et al., 2004). In addition, *Staph. aureus* is widely documented as human pathogen implicated in food-borne diseases through the consumption of milk and milk products in industrialized countries (De Buyser et al., 2001), and *Esch. coli* is just an indicator of faecal contamination and several serotypes (i.e., O157:H7) have been responsible for serious human outbreaks (Keene et al., 1997; Gaulin et al., 2012). Therefore, monitoring programmes of bulk tank milk *Staph. aureus* and *Esch. coli* could be implemented in order to reduce on-farm risk within milk quality and safety assurance programmes in cooperatives of dairy ewe flocks.

On the other hand, some sources of variation, such as breed, milking type, annual season, antibiotic dry therapy, etc., have contributed significantly to variation of specific bulk tank bacterial groups (i.e., psychrotrophs and coliforms) in dairy sheep flocks (de Garnica et al., 2013), but little information is available about the factors influencing the bulk tank *Staph. aureus* and *Esch. coli* variation in this species. The knowledge of the prevalence of these pathogens in bulk tank milk of dairy sheep cooperatives, as well as a wider analysis of the variation of these pathogens and their relationship with BTTBC and BTSCC would enable decision to be made on improving milk quality, farm management practices, and flock udder health within monitoring and traceability programmes.

The objectives of this paper were: (1) to determine the *Staph. aureus* and *Esch. coli* prevalences in bulk tank milk in a large population of dairy sheep flocks, (2) to analyse the relationship among bulk tank *Staph. aureus* and *Esch. coli* on automated BTTBC and BTSCC variables, and (3) to analyse several sources of variation in the prevalence of each organism, such as season, breed, milking system and antibiotic dry therapy, as a basis for the implementation of prevention strategies to minimize the risk in the flocks.

2. Material and methods

2.1. Flocks and sampling

Flocks and bulk tank sampling methodology were described in a previous paper (de Garnica et al., 2013). Briefly, between January and December 2011 a total of 752 bulk tank milk samples, one per season, were collected from 205 dairy sheep flocks (breeds: 172 Spanish Assaf, and 33 Churra; and milking: 182 machine milked, and 23 hand milked) belonging to 2 cooperatives of the Consortium for Ovine Promotion (CPO). The 68 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, two 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 two 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). Milk samples were kept at 4 °C until the bacteriological analysis, which was carried out immediately after arrival in the laboratory in the Department of Food Hygiene and Technology, University of León, Spain. Organisms analysed in unpreserved milk aliquot were *Staph. aureus* and *Esch. coli*. In addition, antibiotic residue screening, BTTBC and BTSCC variables were analysed in the other preserved aliquot in the milk-testing laboratory of the National Center for Animal Breeding and Reproduction in León, Spain.

The information recorded in CPO flocks included the following variation factors: flock, breed, season, milking type (hand and parlour machine milking), annual milk yield per flock, total number of ewes per flock, and antibiotic dry therapy practice in each flock.

2.2. Analytical determinations

As described previously for both organisms in ovine milk (de Garnica et al., 2011), *Esch. coli* detection was carried out using 3M™ Petrifilm™ E.coli/Coliform Count Plates (3M, Minnesota, USA) according to the manufacturer instructions. Plates were incubated at 37 ± 0.5 °C for 24–48 h. Blue colonies with associated gas bubbles were considered as *Esch. coli*. Baird Parker Agar Base supplemented with RPF (Rabbit Plasma Fibrinogen) (Biokar Diagnostics, France) as described in regulation EN ISO 6888–2:1999+A1:2003 (ISO, 2003) was used as the validated method for *Staph. aureus* isolation. In both cases, the volume of sample was 1 ml and the minimum inoculation dilution was 10^{-1}; thus, the detection limit for *Esch. coli* and *Staph. aureus* was 10 CFU/ml.

Antibiotic residues in milk were always checked for β-lactams and tetracycline drugs by Charm ROSA Rapid One Step Assay (Charm Sciences, Inc., Lawrence, MA). Negative results for both drugs were always obtained during the experiment. Total bacterial count was determined with a Bactoscan 8000 instrument (Foss Electric, Hillerød, Denmark) and BTSCC was analysed in a Fossomatic 5000 (A/S N, Foss Electric), both devices were calibrated against ovine standards and subjected to intercomparative trials. Analytical determinations of BTTBC and BTSCC were always performed within 36 h after bulk tank milk collection.

2.3. Statistical analyses

The BTTBC and BTSCC were normalized by log10-transformation. A statistical analysis was carried out using a mixed model according the MIXED procedure of SAS (SAS Institute, 2009). The model used was:

$$Y_{ijklmno} = \mu + B_i + M_j + A_k + S_l + \mathrm{Sa}_m + \mathrm{Ec}_n + b_1 P_{ijklmno} + F_{o(j)} + e_{ijklmno}$$

where $Y_{ijklmno}$ were the dependent variables $\log_{10}$ BTTBC and $\log_{10}$ BTSCC; μ was the intercept; B_i was the fixed effect of breed; M_j was the fixed effect of milking type; A_k was the fixed effect of antibiotic dry therapy; S_l was the fixed effect of annual season, Ec_m was the fixed effect of bulk tank *Esch. coli* presence or absence, Sa_n was the fixed effect of bulk tank *Staph. aureus* presence or absence, $P_{ijklmno}$ was the fixed effect of annual milk yield per flock and ewe in litres of milk per ewe, considered as covariable, being b_1 the slope of regression; $F_{o(j)}$ was the random effect of flock within milking type, and $e_{ijklmno}$ was the random residual effect. Breed effect was divided into 2 levels: Spanish Assaf and Churra. Type of milking effect was divided into 2 levels: hand and parlour machine milking. Dry therapy was divided into 2 levels depending on whether it was carried out in each flock during the previous drying-off or not. Season was divided into 4 periods: winter (months January, February and March), spring (months April, May and June), summer (months July, August and September), and autumn (months October, November and December). *Staph. aureus* was divided into 2 levels: presence and absence, and, similarly, *Esch. coli* was divided into same 2 levels: presence and absence in bulk tank milk. The flock random factor was absorbed in the analysis and only the significance of the fixed effect was shown. Least squares means and test of significance were obtained for *Staph. aureus* and *Esch. coli*.

The variation of the *Staph. aureus* and *Esch. coli* prevalences in bulk tank milk of the flocks was also studied with the SAS CATMOD procedure (SAS Institute, 2009), using the following categorical model:

$$Y \cong B + M + A + S$$

where Y were the *Staph. aureus* and *Esch. coli* positive frequencies (prevalences), calculated as number of positive samples for each organism/total number of samples; B, M, A, and S were the same effects as in the above-mentioned model. Differences between frequencies were tested with a χ^2 analysis, using the SAS FREQ procedure (SAS Institute, 2009).

Flock annual prevalences throughout the year were calculated considering the flocks with at least one sample positive.

This research was carried out in accordance with EU Directive 2010/63/EU for animal experiments.