

# Factors influencing variation of bulk milk antibiotic residue occurrence, somatic cell count, and total bacterial count in dairy sheep flocks

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#### **ABSTRACT**

To study the variations of bulk tank milk variables in dairy ewe flocks and to identify the main target practices and flock groups to improve milk quality and safety, a total of 71,228 records of antibiotic residue (AR) and milk yield and 68,781 records of somatic cell count (SCC) and total bacterial count (TBC) were obtained over 5 yr from the same 209 dairy ewe flocks of the Assaf breed belonging to the Consortium for Ovine Promotion of Castilla-León (Spain). Based on a logistic regression model, year, month, semester, SCC, TBC, dry therapy, and milk yield significantly contributed to AR variation. High SCC was associated with increased AR violations. When antibiotic dry therapy was implemented, AR occurrence was higher than when this practice was not used. A polynomial monthly distribution throughout the year was observed for AR occurrence; the highest values were in autumn, coinciding with low milk yields per flock. Yearly occurrences drastically diminished from 2004 (1.36%) to 2008 (0.30%), probably as a result of effective educational programs. The mixed-model ANOVA of factors influencing variation in SCC and TBC indicated that year, month, AR, dry therapy group, milking type, and year interactions were significant variation factors for SCC and TBC; mathematical model accounted for 74.1 and 35.4% of total variance for each variable, respectively. Differences in management and hygiene practice caused significant SCC and TBC variations among flocks and within flocks throughout the 5-yr study. Over time, continuously dry treated flocks showed lower logSCC (5.80) and logTBC (4.92) than untreated (6.10) and 5.18, respectively) or discontinuously dry treated (6.01) and 5.05, respectively) flocks. Continuously dry treated flocks had lower AR occurrences than did discontinuously dry treated flocks. As a whole, AR occurrence and SCC and TBC bulk tank milk variables can be used for monitoring mammary health and milk hygiene and safety in dairy sheep throughout time.

**Key words:** antibiotic residue, somatic cell count, bacterial count, dairy sheep

#### INTRODUCTION

Antibiotic residue (AR) occurrence, SCC, and total bacterial count (TBC) of bulk tank milk are important to the farmers, cheese manufacturers, and consumers because they are major factors in determining safety and hygienic quality of the final product. Bulk tank parameters have been the target of different legal limits or payment-by-quality schemes proposed by different countries or regions, with obvious repercussion on the marketing of sheep milk. Thus, the European Union establishes both the maximum residue limits of specified veterinary residues (Council Regulation EEC 2377/1990 and subsequent amendments; European Union, 1990) and the limits for TBC (Council Regulation EEC 853/2004; European Union, 2004) in ovine milk, although the European Union has yet to regulate SCC values in ewe milk used for dairy products sold in its region. Castilla-León has about 1.5 million milking ewes and it is the first-largest region in Spain in ewe milk production. The study of AR occurrence, SCC, and TBC variations in milk must be emphasized in the main dairy sheep regions because the production of dairy products starts with raw milk.

Increasing awareness of public health and food safety issues in recent years has led to a greater interest in milk quality. Antibiotics residues are a result of treating dairy sheep with antibiotics and not withholding milk. The treatment of mastitis is probably the most common reason for an increased risk of AR occurrence in dairy farms (Allison, 1985), and a relationship between AR violations and SCC or level of subclinical mastitis has been evidenced in dairy cattle herds (Ruegg and Tabone, 2000; van Schaik et al., 2002). However, little attention has been paid to AR violations in dairy sheep. Yamaki et al. (2004) evidenced a seasonal effect of AR occurrence in raw and heated ewe milk in the Castilla-La Mancha region (Spain). Nevertheless, no

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known studies have empirically investigated the effect of variation factors, such as mastitis, antibiotic therapy, milking type, or milk production, on AR occurrence under field conditions in this species. The identification of risk factors to discriminate between flocks or practices that are at higher risk of AR violations would allow cost-effective targeting of regulatory, educational, and consultative resources in dairy sheep, in a similar way to dairy cattle (Ruegg and Tabone, 2000).

Bulk tank SCC and TBC are principal tools used by technicians and farmers to evaluate udder health as well as the efficiency of production processes and cleaning and sanitation practices. For these variables, the effects of several factors of variation have been recently studied in dairy ewe flocks by Gonzalo et al. (2005, 2006), who also evidenced a significant relationship between both variables. Nevertheless, the association between SCC and AR has not been studied in dairy sheep, and little attention has been paid to relationship between TBC and AR occurrence (Yamaki et al., 2004). In dairy sheep, high SCC levels have been shown to be related to IMI, milk yield losses, and lower quality of dairy products (Marco, 1994; Gonzalo et al., 2002; Raynal-Ljutovac et al., 2007), so preventive mastitis practices, such as antibiotic dry therapy, are frequently used in this species to improve the mammary health and milk yield and quality (Gonzalo et al., 2004b, 2005). In this regard, an attempt should be made to identify and interpret the variation of SCC, TBC, and AR occurrence from an integrated point of view because these variables reflect overall quality of management.

On the other hand, all the above-mentioned studies in dairy sheep were conducted over 1 yr; no known studies examine the variation of these variables over the course of several years. Because of the low occurrence of AR violations, long-term studies are the most suitable for AR occurrence investigation. Over time, observational studies could also allow an optimal knowledge of the effect of some health practices (e.g., dry therapy) or environmental factors (e.g., year) on hygienic variables of bulk tank milk, as well as of interactions between year and other factors of variation. Wider knowledge of AR occurrence, SCC, and TBC variations would enable decisions to be made on improving milk safety and quality, farm management practices, and flock mammary health.

We hypothesized that the study of AR occurrence, SCC, and TBC bulk tank milk variables would be of interest for monitoring the variations in flock mammary health and safety throughout the years. To test this hypothesis, the present study was conducted over 5 yr in 209 Assaf dairy flocks in the Castilla-León region. The objectives of this study were 1) to analyze the main sources of variation in AR occurrence as well as

its relationship with SCC and TBC, and 2) to investigate the influence of the year effect and its interactions with other factors of interest, such as flock, milking type, and antibiotic dry therapy.

#### MATERIALS AND METHODS

A total of 71,228 records of AR and milk yield and 68,781 records of SCC and TBC variables of bulk tank milk were obtained over 5 yr from the same 209 dairy ewe flocks of Assaf breed belonging to the Consortium for Ovine Promotion (CPO) of Castilla-León. All CPO flocks were enrolled in the analysis service of the Dairy Interprofessional Laboratory of Castilla-León. Bulk tank milk samples were collected from the flocks after milk homogenization, preserved with acidiol (3) μL/mL), and kept at 4°C until analysis in the interprofessional laboratory. Somatic cell count was determined with a Fossomatic 5000 instrument (A/S N Foss Electric, Hillerød, Denmark), and TBC was analyzed in a Bactoscan 8000 (A/S N Foss Electric). Antibiotic residues in milk were analyzed by the screening test Eclipse 100ov (ZEU-Inmunotec, Zaragoza, Spain), which is a microbial growth inhibition assay containing spores of Bacillus stearothermophilus var. calidolactis C953 and an acid-base indicator. Incubation was done at 65°C for 3 h. Positive or negative results were obtained by difference of photometric readings at 590 and 650 nm, in comparison with negative control samples. This technique has been previously validated in raw ewe milk (Berruga et al., 2003) and in ewe and goat milk preserved with acidiol (Montero et al., 2005; Roca et al., 2007; Beltrán et al., 2007). Before and during the analysis period, all equipment was subjected to quality control interlaboratory tests by a reference laboratory (Cecalait, Poligny, France). Residue-positive results were periodically confirmed by microbiological multiplate system or chromatography.

In the CPO recording system, the mean number of repeated records per flock and year were 66 to 68 for the variables studied. The information recorded by the CPO veterinary service included the following variation factors: flock, year and sampling month, milking type (hand and machine milking), monthly milk yield per flock, and antibiotic dry therapy (which was given under veterinary supervision; antibiotic treatments at drying-off were always recorded, so the date and each treated ewe lot were known). When dry therapy was performed, complete dry therapy was always carried out in each ewe lot. The time between dry therapy and lambing was >2 mo.

Average herd size was 350 to 400 ewes, which was representative of the Castilla-León region. The reproductive rate in Assaf breed is about 1 lambing/yr. Each

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