Short Communication: Bulk Tank Total Bacterial Count in Dairy Sheep: Factors of Variation and Relationship with Somatic Cell Count

C. Gonzalo,*¹ J. A. Carriedo,* E. Beneitez,† M. T. Juárez,† L. F. De La Fuente,* and F. San Primitivo*

*Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, 24071-León, Spain

†Consorcio de Promoción del Ovino, 49630-Villalpando, Zamora, Spain

ABSTRACT

A total of 9,353 records for bulk tank total bacterial count (TBC) were obtained over 1 yr from 315 dairy ewe flocks belonging to the Sheep Improvement Consortium (CPO) in Castilla-León (Spain). Analysis of variance showed significant effects of flock, breed, month within flock, dry therapy, milking type and installation, and logSCC on logTBC. Flock and month within flock were important variation factors as they accounted for 22.0 and 22.1% of the variance, respectively. Considerable repeatability values were obtained for both random factors. Hand milking and bucket-milking machines elicited highest logTBC (5.31), whereas parlor systems with looped milkline (5.01) elicited the lowest logTBC. The implementation of dry therapy practice (5.12) showed significantly lower logTBC than when not used (5.25). Variability in logTBC among breeds ranged from 5.24 (Awassi) to 5.07 (Churra). However, clinical outbreaks of contagious agalactia did not increase TBC significantly. A statistically significant relationship was found between logTBC and logSCC, the correlation coefficient between the variables being $r = 0.23$. Programs for improving milk hygiene should be implemented for both total bacterial count and somatic cell count variables at the same time.

Key words: bulk tank milk, total bacterial count, milk quality, milking

The presence of microorganisms in milk and dairy products has important ramifications for safety, quality, regulations, and public health. Bulk tank total bacterial count (TBC) is the first and principal tool used by technicians and farmers to evaluate the efficiency of production processes, cleaning and sanitation practices, and to predict the keeping quality and shelf life of milk and dairy products. It is therefore a very useful variable for monitoring and improving flock milk quality in dairy cattle (Jayarao and Wolfgang, 2003). However, little

information is available on TBC in dairy sheep (Muehlherr et al., 2003) and there are no known studies that empirically investigate the effect of variation factors on TBC under field conditions in this species. Indeed, TBC are affected by a number of sources of variation and an attempt should be made to identify them and assess some of their implications in hygiene practices or milk payment schemes. In addition, in dairy cattle there is evidence relating TBC to clinical and subclinical mastitis (Bramley, 1992; Jayarao and Wolfgang, 2003; Phuektes et al., 2003; Zadoks et al., 2004). This association has not been reported in dairy sheep so the relationship between TBC, subclinical mastitis (or SCC), and outbreaks of clinical mastitis (i.e., contagious agalactia in enzootic areas) must be studied in this species. Wider knowledge of TBC and its relationship with udder health would enable decisions to be made on improving milk quality, farm management practices, and flock udder health.

The purpose of this paper was to study factors affecting variation in TBC, in particular flock, sampling month, type and installation of milking, dry therapy practice, clinical outbreaks of contagious agalactia, and breed. Another objective was to study the relationship between TBC and bulk tank SCC in dairy sheep.

A total of 9,353 records for TBC were obtained throughout a complete year from 315 dairy ewe flocks (242 Spanish Assaf, 12 Awassi, 43 Churra, and 18 Castellana flocks) belonging to the Sheep Improvement Consortium (CPO) of Castilla-León (Spain). Castilla-León region has about 1.5 million milking ewes; flock characteristic features of this area have been described (Gonzalo et al., 2005). Teat washing was not done in any flocks before milking, as this is not usual practice in dairy sheep. All CPO flocks were enrolled in the Analysis Service of the Dairy Interprofessional Laboratory of Castilla-León. The mean number of repeated records per flock throughout the year was 30. Total bacterial count was determined by a Bactoscan 8000 (A/S N Foss Electric, Hillerød, Denmark), periodically checked by the reference method (IDF, 1991).

The information recorded by the CPO veterinary service included the following TBC variation factors: flock, breed, sampling month, dry therapy practice, milking

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¹Corresponding author: dp2cga@unileon.es

type (hand and machine milking), and machine-milking installation (buckets, milking parlors with looped milkline, and milking parlors with dead-ended milkline). Antibiotic dry therapy was given under veterinary supervision. In flocks where dry therapy was implemented, all animals were treated during the dry period (complete dry therapy). Clinical outbreaks of contagious agalactia were also reported and *Mycoplasma agalactiae* was isolated in bulk tank milk.

A mixed model, in which the flock and month within flock were random factors and the other effects fixed, was used. The PROC MIXED procedure of SAS (SAS Institute, 1998) was followed, according to the model below:

$$Y_{ijklmno} = \mu + B_i + H_{j(i)} + T_k + D_l + M_{m(ij)} + A_n + b x_{ijklmno} + e_{ijklmno}$$

where $Y_{ijklmno}$ was the dependent variable logTBC, B_i was the fixed effect of breed, $H_{j(i)}$ was the random effect of flock nested within breed, T_k was the fixed effect of type and installation of milking, D_l was the fixed effect of dry therapy, $M_{m(ij)}$ was the random effect of month within flock, A_n was the fixed effect of clinical outbreak of contagious agalactia, b was the slope of regression corresponding to the covariable logSCC, and $e_{ijklmno}$ was the random residual effect. Breed effect was divided into 4 levels: Spanish Assaf, Awassi, Churra, and Castellana. Effect of type and installation of milking was divided into 4 levels: hand milking, bucket, parlor with looped milkline, and parlor with dead-ended milkline. Dry therapy was divided into 2 levels depending on whether it was carried out in each flock during the previous drying-off or not. The contagious agalactia effect was divided into 2 levels: presence or absence of a clinical outbreak with *M. agalactiae* in bulk tank milk.

Estimation of variance components was made for random factors of the model according to REML methodology (VARCOMP procedure; SAS Institute, 1998).

To obtain TBC monthly variations throughout the year, a second statistical analysis was carried out on the same set of data, considering the same factors included in the prior model, although in this case, the effect of the month was considered as a fixed factor rather than a random factor within flock as before.

Mean logTBC was 5.13 ± 0.005 (geometric mean: 135×10^3 cfu/mL). This value was higher than that reported for cow bulk tank milk (Jayarao and Wolfgang, 2003), but was much lower than the limits established by the European Union (Directive 94/71/EEC) for sheep milk used in dairy products ($1,500 \times 10^3$ cfu/mL in milk subjected to heat treatment and 500×10^3 cfu/mL for milk not subjected to heat treatment). Lower individual milk

yields and the absence of teat-washing practice before milking might partially explain greater TBC values in dairy sheep compared with dairy cattle.

Table 1 shows the ANOVA of the variation factors of the TBC variable from the mixed model used. Breed, milking, and dry therapy contributed significantly ($P < 0.05$ to 0.001) to variation of TBC. The components of variance for the random factors of the mixed model and the percentages of variance explained are also shown in Table 1. Flock accounted for 22.0% of the total variance and was, therefore, an important source of variation in TBC. Thus, differences in management and hygiene practice in flocks would cause important TBC variations amongst flocks. Similarly, month within flock was also a relevant factor of variation in TBC (22.1% of total variance) probably due to different hygiene and management conditions throughout the year in each flock. In this sense, not only does hygiene vary among flocks, but also within flock. So, depending on the production level of the flock and availability of pastures and agricultural by-products, there are alternating periods of confinement and grazing throughout the year. These aspects are specific to each flock and they even vary across years within flock. Therefore, inclusion of confinement vs. grazing in the mathematical model was not possible. In addition, because of the reproductive singularity of dairy sheep flocks in Castilla-León (Gonzalo et al., 2005), month within flock could also explain, although only in part, differences in stage of lactation within flocks, due to lambing concentration. Nevertheless, this aspect could be debatable according to specific reproductive features in each flock. When month was considered as fixed effect and not within flock in the statistical model, the autumn months (log TBC: 5.24 to 5.40, and geometric means: 175 to 257×10^3 cfu/mL) showed higher values ($P < 0.05$) than the remaining months (5.05 to 5.22, and 113 to 169×10^3 cfu/mL).

Repeatability of logTBC for the random factor month within flock was high (0.50). However, the repeatability of random factor flock corresponding to repeated observations of TBC within flock was lower (0.25), indicating some variation in this variable for each flock throughout the year (Table 1). Considering the value for both repeatabilities, the actual recording system of TBC is adequate for monitoring flock hygiene and for being used as a criterion for milk payment schemes in dairy sheep, though a larger number of samples per flock and year would improve the system.

For milking comparisons (Table 2), hand milking (logTBC: 5.31, and geometric mean: 202×10^3 cfu/mL) and bucket-milking machines (5.31 and 206×10^3 cfu/mL) had less desirable hygiene conditions ($P < 0.05$) than milking parlor systems. Within the latter, the sys-

Table 1. Test of significance of fixed effects of bulk tank total bacterial count from mixed model, components of variance, and repeatabilities for the random factors of the mixed model

Variation factor	df	F	Variance	Variance explained (%) ¹	Repeatability ²
Breed	3	3.06*	—	—	—
Milking	3	22.80***	—	—	—
Dry therapy	1	14.51***	—	—	—
Contagious agalactia	1	1.00 ^{NS}	—	—	—
Covariable logSCC	1	27.54***	—	—	—
Flock within breed ^{3,5}	—	—	0.047	21.98	0.25
Month within flock ^{4,5}	—	—	0.047	22.12	0.50
Residual	—	—	0.095	44.70	—
Total	9,352	—	0.2125	—	—

¹Percentages of variance explained were calculated by dividing the corresponding components of variance by the total variance.

²Bulk tank total bacterial count repeatability for flock factor was defined as the ratio of flock variance to the sum of the residual, month within flock and flock variances, and repeatability of month within flock as the ratio of the sum of flock and month variances to the sum of the residual, month within flock and flock variances.

³Levels of random factor flock: 315.

⁴Levels of random factor month within flock: 3780.

⁵Random factors absorbed in the model.

* $P < 0.05$; *** $P < 0.001$; ^{NS}Nonsignificant.

tems with dead-ended milkline (5.10 , and 125×10^3 cfu/mL) had higher TBC ($P < 0.05$) than the looped milkline systems (5.01 , and 102×10^3 cfu/mL). These results reflect the inferior hygiene conditions of hand and bucket milking, systems that make it more difficult for farmers to reach milk quality standards as measured by TBC. Bucket-milking systems are not used in milking parlors where conditions are more suitable for hygiene milking. In addition, the greatest difficulties for cleaning in parlors with dead-ended milklines could explain the higher TBC values in comparison with looped milklines.

Dry therapy was a very important variation factor of TBC; when dry therapy was implemented TBC (5.12 , and 133×10^3 cfu/mL) was lower ($P < 0.001$) than when this practice was not used (5.25 , and 176×10^3 cfu/mL). Dry therapy was generally associated with improved milking hygiene in subsequent lactation, so this practice is an efficient method of reducing IMI prevalence (Gonzalo et al., 2004) and improving the hygiene and health quality of milk.

Breed had a significant effect ($P < 0.05$) on TBC. The highest values (log TBC and geometric mean) were for Awassi (5.24 and 176×10^3 cfu/mL) characterized mainly by management system based on confinement housing, whereas the other breeds with more extensive systems, showed lower values (5.07 to 5.20 , and 117 to 157×10^3 cfu/mL) than did Awassi. These results were consistent with greater TBC values found in confinement vs. grazing systems in dairy cattle (Goldberg et al., 1992).

Clinical outbreaks of contagious agalactia did not increase TBC ($P > 0.05$). This enzootic disease is associated with large increases in bulk tank SCC (Bergonier et al., 1996; Gonzalo et al., 2005), and is a serious hindrance for optimizing SCC and mastitis control strategies (Madanat et al., 2001). However, TBC was not a useful variable for diagnosing contagious agalactia. *Mycoplasma agalactiae* is an organism that does not have a bacterial wall; thus, it requires specific culture media and conditions for growth (Razin et al., 1998). The lack of cell wall could account for the emission of

Table 2. Least squares means of bulk tank bacterial count from mixed model corresponding to milking effect

Variable	Hand milking	Machine milking		
		Bucket	Dead-ended line	Looped line
Log TBC (SE)	5.31 ^a (0.04)	5.31 ^a (0.09)	5.10 ^b (0.04)	5.01 ^c (0.04)
TBC ¹	202	206	125	102

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹Geometric mean of total bacterial count (TBC).

weak pulses not counted by the Bactoscan because of faint fluorescent staining of the mycoplasmal cells. Other authors (Phuektes et al., 2003) were not able to find a relationship between presence of *Staphylococcus aureus*, *Streptococcus dysgalactiae*, or *Streptococcus uberis* and TBC or SCC in bulk tank milk of dairy cattle; however, presence of *Streptococcus agalactiae* was associated with high SCC and TBC. Consequently, these associations should be specifically studied for each pathogen.

Parameter b ($\pm$ SE) of the regression of logSCC on logTBC in the mixed model was significant ($b = 0.119 \pm 0.023$; $P < 0.001$), and the correlation coefficient between these variables was $r = 0.23$ ($P < 0.001$), indicating that high SCC in bulk tank milk are associated with high TBC. The relationship between SCC and sub-clinical mastitis has been clearly demonstrated in dairy sheep (González-Rodríguez et al., 1995; Gonzalo et al., 2002; Marco, 1994), so high IMI prevalence would be related to high TBC values. In the same way, poor flock and milking hygiene could favor a higher frequency of mammary infections and high TBC values. Therefore, quality hygiene and quality health of bulk tank milk are complementary aspects, which should be studied at the same time.

In summary, total bacterial count can be used for monitoring hygiene in dairy ewe flocks and as a basis for milk payment schemes. The relatively low TBC recorded compared with European Union regulations suggests the presence of acceptable hygiene practices in dairy sheep flocks. Dry therapy practice and milking in parlor systems were associated with low TBC. Total bacterial count had a statistically significant correlation with bulk tank SCC, so the implementation of programs for improving hygiene and health quality in bulk tank milk would be more efficient if both variables are considered.

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