# Bacteriological Properties Of Udder Surface And Milk Samples From Sheep Farms In Hungary

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# Abstract

Background Ewe milk due to its beneficial composition and properties contributes to the growth of microorganisms. The primary prerequisite for making high-quality sheep milk product is the production of high-quality raw sheep milk by dairy farms. Thus, the aim of this study was to examine the bacteriological properties of the udder surface (US), individual ewe raw milk (IERM) and bulk tank milk (BTM) samples from sheep farms in Hungary. Methods Seventy-seven US, seventy-seven IERM, and ten BTM samples were examined from March 2018 to April 2019. Total plate count (TPC), Enterobacteriaceae count (EBC), Escherichia coli count (ECC), Staphylococcus aureus count (SAC), lactic acid bacteria count (LAB) and psychrotrophic bacteria count (PBC) of different ewe breeds were examined according to ISO standards in four Hungarian sheep farms. The differences in the microbiological status between the breed and farm were considered. Results High counts of TPC ($3.2\pm1.1$ lg cfu/cm 2 ) and EBC ($2.4\pm0.7$ lg cfu/cm 2 ) were found in Cigája breed of US samples in farm1. TPC of US sample of Lacaune (farm2), Lacaune (farm3) and British milk sheep was $2.7\pm0.8$, $2.2\pm0.5$ and $2.4\pm0.4$ lg cfu/cm 2 , respectively. The mean value of TPC of IERM of Merino, Cigája and Dorper breeds in farm1 was $2.8\pm1.2$, $3.5\pm1.2$ and $3.3\pm0.7$ lg cfu/ml, respectively. There was significant difference ($p<0.05$) between Merino and Cigája breeds for TPC. Although there was no significant difference ($p<0.05$) between breeds for EBC, the mean of EBC of IERM samples from Dorper breed was highest in farm1. Comparatively low TPC of IERM of British milk sheep breed was recorded. The mean of TPC of BTM was $7.4\pm0.6$, $6.3\pm0.4$ and $5.2\pm0.1$ in farm2, 3 and 4, respectively. Both LAB and PBC were high in BTM of Lacaune breed. Except for BTM, SAC and ECC were not detected in most US and IERM samples. Conclusion The presence of microorganisms in BTM above the limit indicates high microbial contamination due to poor hygienic conditions during milking and milk handling. Practicing very good hygiene principles at the farms, in handling and transportation of milk, is a must.

# Background

Dairy sheep industry is a promising branch of livestock production in Hungary. According to the report of Hungarian Central Statistical Office (KSH), the country has 798, 000 heads of ewe in 2018. In the country, most of ewe population milked belongs to Merino breeds, however, there are some milk breeds like Lacaune, Awassi, milking Tsigai and British milk sheep [1]. Data from Faostat [2] revealed that Hungary takes 15th place from EU member countries with the sheep milk production of 1149 tonnes in the year of 2017. The interest for ewe milk in Europe is increasing from time to time. Similarly, in Hungary it was observed that there was yearly change in sheep milk production [2]. However, ewe milk is an excellent media for the growth of microorganisms due to the high content of fat, protein, total solids, essential vitamins and minerals [3].

Milk is virtually sterile when it is synthesized and secreted into a healthy alveoli of ewe mammary gland. However, the exterior of ewe's udder can contribute bacteria that are associated with the skin of animal and from the environment in which animals are housed and milked [3]. The microbiological quality of the sheep milk can be affected by contamination during and after milking, method of milking, breed, season

and the hygiene of farms [4]. According to the Regulation (EC) 853/2004 of the European Parliament and the Council [5], the total count of microorganisms is a basic and mandatory indicator for evaluation of raw sheep milk quality. TPC of ewe milk that will undergo pasteurization before processing must not exceed $1.5 \times 10^6$ cfu/ml and shall not exceed TPC of $5 \times 10^5$ cfu/ml if the milk is intended for processing without heat treatment [5].

Unpasteurized ewe raw milk contains pathogenic and non-pathogenic bacterial population. Ewe milk is dominated by lactic acid bacteria (LAB) [6]. One of the main fermentation products of the metabolism of carbohydrates, lactic acid, was produced by LAB [7]. The consumption of raw ewe milk has a risk for the consumer, due to the possible presence of human pathogenic organisms in the raw ewe milk [8]. Oliver et al. [9] from USA reported that raw milk could be contaminated by pathogenic microorganisms as clearly shown by numerous epidemiological studies. Ewe milk is a source of undesirable bacteria like Enterobacteriaceae, Escherichia coli, psychotrophic bacteria and Staphylococcus aureus [10]. Apart from Hungary, there were literature which dealt with the microbiological quality of ewe milk, especially in BTM in different ewe breeds at different places and time. For instance, the study by Ombarak and Elbagory [11] from Egypt pointed out that the mean value of total bacterial count was $2.04 \times 10^6$ cfu/ml, Enterobacteriaceae was $1.67 \times 10^5$ cfu/ml and S. aureus was $6.67 \times 10^4$ cfu/ml in raw ewe milk.

This study was based on the fact that bacteriological quality studies on sheep milk have so far been relatively neglected although the production (and the interest towards increased production) is showing an upward trend worldwide. This is also true for Hungary, where there is a paucity of information on bacteriological analysis of raw sheep milk. Therefore, this study was designed to study the bacteriological properties of udder surface, individual ewe raw milk and bulk tank milk samples of Merino, Tsigai, Dorper, Lacaune and British milk sheep breeds in four Hungarian dairy sheep farms in Hajdú-Bihar and Jász-Nagykun-Szolnok Counties.

# Results

## Bacteriological quality of udder surface samples

The result revealed that the mean value of TPC was 2.5±1.0, 3.2±1.1 and 2.5±0.8 lg cfu/cm$^2$ in US samples of Merino, Tsigai and Dorper breed from F1, respectively (Table 2). There was a significant difference (p<0.05) between Merino and Tsigai, Tsigai and Dorper breeds for TPC. The mean of TPC of US samples from Tsigai breed was significantly (p<0.05) higher than TPC of US samples from Merino and Dorper breeds. In the case of F2 and F3, the mean value of Lacaune breed was 2.7±0.8 and 2.2±0.5 lg cfu/cm$^2$, respectively. The mean of TPC of US samples from F2 was significantly (p<0.05) higher than F3. In the case of F4, the mean of TPC of US samples of British milk sheep was 2.4±0.4 lg cfu/cm$^2$.

The mean value EBC of US samples was 0.9±0.3, 2.4±0.7 and 1.7±0.2 lg cfu/cm$^2$ in Merino, Tsigai and Dorper breeds from F1, respectively (Table 2). There was significant difference (p<0.05) between US samples taken from Merino and Tsigai breeds for EBC. The mean of EBC of US samples originated from

Tsigai breed was significantly ($p<0.05$) higher than EBC of US samples from Merino breeds. In the case of F2 and F3, the mean EBC of US samples of Lacaune ewe was 1.0±0.6 and 1.0±0.1 lg cfu/cm$^2$, respectively (Table 2). There was no significant difference between mean value of EBC of US sample from F2 and F3. SAC and ECC were <1 lg cfu/cm$^2$ in US of F1, not examined in US samples from F2 and not detected from F3 and F4 (Table 2).

## Bacteriological quality of ewe milk

The mean values of TPC were 2.8±1.2, 3.5±1.2 and 3.3±0.7 lg cfu/ml in IERM samples of Merino, Tsigai and Dorper breeds from F1, respectively (Table 3). There was significant difference ($p<0.05$) between Merino and Tsigai breeds for TPC. The mean of TPC of IERM samples from Tsigai breed was significantly ($p<0.05$) higher than TPC of IERM samples from Merino breeds. In the case of F2 and F3, the mean TPC of Lacaune breed was 3.3±1.0 and 3.5±0.9 lg cfu/ml, respectively (Table 3). Even though, there was no significant ($p>0.05$) difference between two farms, the mean of TPC of IERM samples from F3 was higher than F2. The mean of TPC of IERM samples from British milk sheep breed in F4 was 1.8±0.4 lg cfu/ml.

The mean values EBC of IERM samples were 1.0±0.3, 0.0±0.0 and1.4±0.0 lg cfu/ml from Merino, Tsigai and Dorper breeds in F1, respectively (Table 3). There was no significant difference ($p<0.05$) between breeds for EBC. However, the mean of EBC of IERM samples from Dorper breed was highest. In the case of F2 and F3, the mean EBC from Lacaune breed was 2.2±1.1 and 2.2±0.0 lg cfu/ml, respectively (Table 3). The mean of EBC of IERM samples of British milk sheep breed in F4 was 1.4±0.0 lg cfu/ml. SAC was 2.6±0.7 and 2.8±0.3 lg cfu/ml in in IERM F3 and F4, respectively (Table 3). ECC was not detected in IERM from F1 and F4 (Table 3). Regardless of the breed, LAB was the same in IERM of F3 and F4 (Table 3).

The mean of TPC and EBC was 7.4±0.6 and 5.1±0.9 lg cfu/ml in BTM in F2, respectively (Table 4). TPC, EBC, SAC, ECC, LAB and PBC was 6.3±0.4, 5.6±0.9, 3.4±0.6, 2.9±0.5, 6.7±0.4 and 3.9±1.1 lg cfu/ml in BTM in F3, respectively (Table 4). There was no significant difference between F2 and F3 of TPC and EBC of BTM. The mean of TPC, EBC, ECC, LAB and PBC was 5.2±0.1, 3.9±0.0, 3.8±0.9, 4.2±0.2 and 3.6±0.2 in BTM of F4, respectively (Table 4).

The bacteriological count results by years of sample collection are shown in table 5. The mean of TPC of US samples examined in all breeds has got significant ($p<0.05$) difference between years (Table 5). A significantly higher value was recorded in 2018 than 2019 for TPC of US samples. Also, there was significant ($p<0.05$) difference between years for EBC of US samples taken from Tsigai and Dorper breeds. In the case of IERM, TPC was significantly ($p<0.05$) different between years in Merino and Tsigai (Table 5). A significantly higher value was recorded in 2018 than in 2019.

## Correlation between microbiological parameters

Linear correlation coefficient of Pearson was calculated to evaluate the correlation between US and IERM for TBC and EBC (Table 6). Moderate and positive correlations were observed between EBC$^{IERM}$ and

EBC$^{US}$ (r = 0.56). Weak and positive correlations were observed between TPC$^{IERM}$ and EBC$^{IERM}$ (r = 0.29), TPC$^{US}$ and EBC$^{IERM}$ (r = 0.27), TPC$^{US}$ and EBC$^{US}$ (r = 0.32). Very weak and positive correlations were observed between TPC$^{IERM}$ and TPC$^{US}$ (r= 0.18), TPC$^{IERM}$ and EBC$^{US}$ (r = 0.02) (Table 6).

## Discussion

Natural reservoirs of bacteria like the body of the animal, especially, the udder surface of sheep contributes the contamination bacteria in raw milk [3]. The udder surface of ewe certainly become contaminated with manure and mud while they are lying. This was one of reasons US samples were examined. Also, the diameter of US was easily measured during sampling. The higher value of TPC of US samples in Tsigai breed than Merino and Dorper breeds is might be due to the difference in the degree of udder surface dirtiness with manure and dust particles. In this study, the mean of TPC of US samples from F2 was significantly (p<0.05) higher than F3. This could be related to factors such as the location of the farm, hygienic condition of farm and handling of animals. The mean of EBC of US in Tsigai breed was significantly (p<0.05) higher than Merino breeds. This was maybe due to a hairy udder surface which stick with dust and feaces in the case of Tsigai breeds. In general, the low EBC of US samples indicates a good hygienic condition of ewe housing. Comparable data on a bacteriological load of US samples of sheep breeds are rare in literature, and this study is the first dealing with this specific parameter in the studied area.

In the present study, the significant difference (p<0.05) between Merino and Tsigai breed for TPC in IERM was observed. This finding was in contrast with the finding of Alexopoulos et al. [4] reported that the difference between ewe breed was not significant (p>0.05). Even though, the difference between F2 and F3 was not significant (p>0.05), the mean of TPC of IERM samples from F3 higher than F2. This was might be because of hand milking and absence of pre-milking disinfection in F3. This finding differed from the report of Bytyqi et al. [12] from Kosovo who reported that the farm had a significant effect on TPC. Along with the poor hygienic conditions of hand milking, the hand can act as a vector for transmission of environmental and contagious pathogens, increasing their counts in milk [13]. Comparatively low TPC of IERM of British milk sheep breed could be because of extremely hardy and strong characteristics of British milk sheep. The hygienic practices during milking, raw milk and milk products quality can be indicated by examining total plate count of bacteria [14].

The higher value of EBC (1.4±0.0 lg cfu/ml) of IERM was found in Dorper breeds in F1 (Table 3). Our result was lower than the finding of Ombarak and Elbagory [11] in Egypt, which was 5.2 lg cfu/ml in raw ewe milk. SAC was <1 lg cfu/ml in F1 and not detected in F2 (Table 3). However, Alexopoulos et al. [4] reported that S. aureus was detected in raw ewe milk at an average level of 3.94 lg cfu/ml. S.aureus can easily grow in ewe milk due to an excellent substrates of it. The mean value of LAB (3.3 lg cuf/ml) was the same in IERM of Lacaune and British milk sheep (Table 3). This result was lower than the finding of Kalhotka et al. [15] from Czech Republic which was 6.4 lg cfu/ml in raw milk of Lacaune breed.

The higher values of EBC in BTM is an indication of the possibility of bacterial contamination via the udder, by milking equipment or faeces [6]. Increased numbers of *Enterobacteriaceae* in BTM can also occur when *Enterobacteriaceae* grow on residual milk left in poorly sanitized milking equipment. The high count of LAB in BTM in F3, could be one of the reasons for the better quality of ewe's milk compared to the cow's milk. The PBC mean value in this study was less than the value reported by de Garnica et al. [13] from Spain, which was 5.7 lg/cfu in BTM of sheep. Storing milk more than 24 hr had an impact on PBC results leading to higher microbial counts.

In this study, the microbiological quality of ewe milk from BTM based on TPC values, except TPC of F4, were at unacceptable levels according to European Parliament and of the Council 853/2004 standard; TPC of ewe milk that will undergo pasteurization before processing must not exceed 6.2 lg cfu/ml. Thus the milk originated from the farms, where this study was undertaken, should be pasteurized before fermentation since the highest TPC count reached 7.4±06. Our result was higher than the finding of Zweifel et al. [16] from Switzerland which was 4.79 lg cfu/ml of TPC in BTM. Besides, mean values of TPC were higher than the report of Alexopoulos et al. [4] that the average TPC of 5.48 lg cfu/ml in Greece. Therefore, our finding indicated that there is contamination of milk during milking and milk handling. At present, there is no legislative limit for the EBC and coagulase-negative staphylococci in ewe milk, they are considered to be an indicators of hygienic circumstances in ewe milk production [17].

The mean of TPC of US from all breeds had significant ($p<0.05$) difference between years (Table 5). The explanation may be the dependence of bacterial load on the season and lactation dynamics. Also, there was significant ($p<0.05$) difference between years for EBC of US from Tsigai and Dorper breeds. The higher value of EBC during 2018 might be supported by higher contamination of US of ewe. It could be due to the rising awareness of the sheep farmers about the urgent need for increasing the hygiene standards during the milking process. In the case of IERM, TPC was significantly ($p<0.05$) different between years in Merino and Tsigai (Table 5). Similarly, the finding by Gonzalo et al. [18] from Spain confirmed that year was significant source of variation for TBC in BTM. In fact, from the results, it is evident that the hygienic milk quality has improved during 2019 compared to 2018. Moderate and positive correlations were observed between EBC$^{IERM}$ and EBC$^{US}$ (r = 0.56). This was might be the evidence that the environmental sources, such as dirty bed and contaminated feed are predisposing factors for the presence of this bacteria in raw milk In general, poor cleaning practices in the flocks tend to results in higher bacterial load in the milk.

## Conclusions

In conclusion, there was no significant difference between ewe breeds for *Enterobacteriaceae* count of individual ewe raw milk. However, total plate count of individual ewe raw milk samples from farm1 was significantly different between breeds. Therefore, the breed affected the bacteriological status of individual ewe raw milk samples. Total plate count of individual ewe raw milk samples from Lacaune breeds has got no significance difference between farms. Hence, the farm had no significant effect on the bacteriological status of raw milk samples. There was a significant difference between the year of

examination in the case of total plate count of individual ewe raw milk. Therefore, the year had an effect on the bacteriological status of individual ewe raw milk samples. Relatively low *Enterobacteriaceae* count of udder surface samples indicates a good hygienic condition of ewe housing. The relatively low bacterial count of individual ewe raw milk indicates good health condition of ewes. The number of bacterial count in bulk tank milk at the examined farms was high and urgent steps must be taken to improve milk quality by the respect of strict hygienic measures.

# Methods

## Animals and management of the farms

This study was conducted between March 2018 and April 2019 on several times at four Hungarian sheep farms. The farm1 (F1), farm2 (F2) and farm3 (F3) located in Hajdú-Bihar County and farm4 (F4) located in Jász-Nagykun-Szolnok County. The flock was composed of Merino, Tsigai and Dorper breed in F1, Lacaune in F2 and F3 and British milk sheep in F4. The main characteristics of the farms were summarized in table 1 below.

## Sampling and sample preparation

During the experiment, 42 US and 42 IERM from F1, 15 US, 15 IERM and 6 BTM from F2, 10 US, 10 IERM and 3 BTM from F3 and 10 US, 10 IERM and 1 BTM samples from F4 were collected and examined at different times. A total of 77 US, 77 IERM and 10 BTM samples were examined. All the samples were collected in the early morning from an unmilked ewes during milking activity performed by farmers. At first, US samples were collected from 20 cm$^2$ area of the udder by transport swabs moistened with a solution of 0.1% peptone (Merck Kft., Hungary) and 0.9% NaCl (VWR International Ltd., Hungary). Before milk sampling, ewe teats were cleaned with cotton soaked in 70% ethanol (Molar chemicals Kft., Hungary). Samples were delivered to the laboratory of microbiology at Institute of Food Science, University of Debrecen in plastic bag with ice packs at less than 4 $^o$C within 30 minutes to 1 hour of collection and examined immediately.

## Bacteriological examination

Upon arrival at the laboratory, milk samples were kept in the refrigerator until examinations. Nine ml peptone water was prepared for decimal dilution as described by Petróczki et al. [19]. Total plate count (TPC) was performed according to MSZ EN ISO 4833-1[20] as described by Tonamo et al. [21]. *Enterobacteriaceae* count (EBC) was performed on Violet Red Bile Glucose (VRBG) agar (Biolab Ltd., Hungary) following MSZ EN ISO 21528-2 [22]. *Escherichia coli* count (ECC) was performed according to ISO 16649-2:2001 on Tryptone Bile Glucoronide (TBX) agar (Biolab Ltd., Hungary) medium and was incubated at 37 $^o$C for 24 hrs. All plates were incubated at 37 $^o$C for 24 hrs. Culturing and Latex agglutination test of *Staphylococcus aureus* were performed according to MSZ EN ISO 6888-1 [23] standard as described by Petróczki et al. [19]. Lactic acid bacteria count (LAB) was examined according to MSZ EN ISO 15214 [24] standard. Using the pour plate method, 1 ml of the test sample was inoculated

into the Petri dish before pouring over the molten de Man, Rogosa and Sharpe agar (MRS). The incubation was at 30 °C for 72 hrs. Psychrotrophic bacteria count (PBC) was examined on plate count agar medium (Biolab Ltd, Hungary) according to MSZ ISO 17410-2 [25]. Plates were incubated at 7 °C for 10 days. All samples were plated in duplicate. Accordingly, bacteriological properties of US samples were carried out and the results were divided by 20.

## Statistical analysis

Data on bacterial count were entered into MS Excel sheet and log-transformed before analysis. SPSS version 20 [26] was used for analysis. T-tests, one-way ANOVA and non-parametric test (Kruskal-Wallis test) were used to check for differences on mean values by ewe breed groups, year and farms. Regarding comparison; the mean value from three breeds in F1 was compared, between years (2018 and 2019) in F1, then between farms (F2 and F3), where the Lacaune breed performed differently and F4 on their own. Linear correlation coefficient of Pearson between bacteriological parameters of US and IERM samples was calculated. Differences between ewe breed, farm and year were considered to be significant at $p < 0.05$.

# Abbreviations

ANOVA: analysis of variances; BMS: British milk sheep; BP: Baird Parker; BTM: bulk tank milk; CFU: colony forming unit; EBC: *Enterobacteriaceae* count; ECC: *Escherichia coli* count; IERM: individual ewe raw milk; ISO: International Organization for Standardization; LAB: Lactic acid bacteria; MRS: de Man, Rogosa and Sharpe agar; ND: not detected; NT: not tested; PBC: Psychrotrophic bacteria count; PCA: plate count agar; SAC: *Staphylococcus aureus* count; SPSS: statistical package for the social sciences; TBX: Tryptone Bile Glucoronide; TPC: total plate count; US: udder surface; VRBG: Violet Red Bile Glucose.

# Declarations

## Ethics approval and consent to participate

The milk sampling was carried out following the Hungarian Sheep and Goat Breeders Association Performance Testing Codex (https://mjksz.hu/sites/default/files/pdf/juhteljesitmenyvizsgalatikodex2013_0.pdf). Milk samples were collected from private farms during routine milking by farmers. The Hungarian regulation/decree No. 40/2013 (II. 14.) of government on animal testing had set very compressive decree on experimental animals handling in chapter 1 paragraph 1 (2) and (4) (http://njt.hu/). Based on this decree, the ethics approval was unnecessary for this study. Dairy sheep farm owners and managers were also asked for their consent verbally before the start of the sampling. Verbal consent was taken because the sampling was not invasive to the animals according to the Hungarian Sheep and Goat Breeders Association Performance Testing Codex, and Hungarian Government on animal testing decree No. 40/2013 (II. 14.) Chapter 1 paragraph 1 (2) and (4).

## Consent for publication

Not applicable

## Availability of data and materials

All data generated and analysed during this study are included in this article. Further information on the data can be obtained from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

AT, IK and FP collected all the required data; AT performed the laboratory works; IK and FP participated in coordination and supervision; IK, FP and AT designed the study and drafted the manuscript; AT analysed and interpreted the data; IK, CL and FP critically and substantially revised the manuscript. All authors read and approved the final manuscript.

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## Tables

**Table 1** Characteristics of dairy sheep farms

| Farms | Breed of ewe | Type of milking | Grazing | Housing | Pre/post-milking disinfection |
|---|---|---|---|---|---|
| F1 | Merino, Tsigai and Dorper | No milking | Yes | Deep litter | No |
| F2 | Lacaune | Milking parlour | Yes | Deep litter | Pre-milking disinfection |
| F3 | Lacaune | Hand milking | Yes | Deep litter | No |
| F4 | British milk sheep | Milking parlour | Yes | Deep litter | No |

**Table 2** Bacteriological status of udder surface samples according to the farm and breed

| Farm | Breed | Microbiological count (lg cfu/cm$^2$) mean ± standard deviation | | | |
|---|---|---|---|---|---|
| | | TPC | EBC | SAC | ECC |
| F1 | Merino | 2.5±1.0$^a$ | 0.9±0.3$^a$ | <1.0 | <1.0 |
| | Tsigai | 3.2±1.1$^b$ | 2.4±0.7$^b$ | <1.0 | <1.0 |
| | Dorper | 2.5±0.8$^a$ | 1.7±0.2$^{ab}$ | <1.0 | <1.0 |
| F2 | Lacaune | 2.7±0.8$^a$ | 1.0±0.6$^a$ | ND | NT |
| F3 | Lacaune | 2.2±0.5$^b$ | 1.0±0.1$^a$ | ND | ND |
| F4 | BMS | 2.4±0.4 | 0.5±0.3 | ND | ND |

$^{ab}$Mean value in the column with different letters are significantly (p< 0.05) different

TPC: total plate count, EBC: *Enterobacteriaceae* count, SAC: *Staphylococcus aureus* count, ECC: *Escherichia coli* count, BMS: British milk sheep, ND: not detected, NT: not tested

**Table 3** Bacteriological status of individual ewe milk samples according to the farm and breed

| Farm | Breed | Microbiological count (lg cfu/ml) mean ± standard deviation | | | | |
|---|---|---|---|---|---|---|
| | | TPC | EBC | SAC | ECC | LAB |
| F1 | Merino | 2.8±1.2$^a$ | 1.0±0.3$^a$ | <1.0 | ND | NT |
| | Tsigai | 3.5±1.2$^b$ | 0.0±0.0$^a$ | <1.0 | ND | NT |
| | Dorper | 3.3±0.7$^{ab}$ | 1.4±0.0$^a$ | <1.0 | ND | NT |
| F2 | Lacaune | 3.3±1.0$^a$ | 2.2±1.1$^a$ | ND | NT | NT |
| F3 | Lacaune | 3.5±0.9$^a$ | 2.2±0.0$^a$ | 2.6±0.7 | <1.0 | 3.3±1.0 |
| F4 | BMS | 1.8±0.4 | 1.4±0.0 | 2.8±0.3 | ND | 3.3±0.6 |

$^{ab}$Mean value in the column with different letters are significantly (p< 0.05) different

TPC: total plate count, EBC: *Enterobacteriaceae* count, SAC: *Staphylococcus aureus* count, ECC: *Escherichia coli* count, LAB: lactic acid bacteria, ND: not detected, NT: not tested, BMS: British milk sheep

**Table 4** Bacteriological status of bulk tank milk

| Farm | Breed | Microbiological count (lg cfu/ml) mean ± standard deviation | | | | | |
|---|---|---|---|---|---|---|---|
| | | TPC | EBC | SAC | ECC | LAB | PBC |
| F2 | Lacaune | 7.4±0.6$^a$ | 5.1±0.9$^a$ | ND | NT | NT | NT |
| F3 | Lacaune | 6.3±0.4$^a$ | 5.6±0.9$^a$ | 3.4±0.6 | 2.9±0.5 | 6.7±0.4 | 3.9±1.1 |
| F4 | BMS | 5.2±0.1 | 3.9±0.0 | ND | 3.8±0.9 | 4.2±0.2 | 3.6±0.2 |

$^{ab}$Mean value in the column with different letters are significantly (p< 0.05) different

TPC: total plate count, EBC: *Enterobacteriaceae* count, SAC: *Staphylococcus aureus* count, ECC: *Escherichia coli* count, LAB: lactic acid bacteria, PBC: psychrotrophic bacteria count, ND: not detected, NT: not tested, BMS: British milk sheep

**Table 5** Bacteriological status of US (lg cfu/cm$^2$) and IERM (lg cfu/ml) samples according to the year of examination (2018 and 2019) in case of farm1

| Origin of sample | Parameters (Mean ± SD | Merino | | Tsigai | | Dorper | |
|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| US | TPC | 3.8±0.5$^a$ | 1.9±0.4$^b$ | 4.0±0.7$^a$ | 2.1±0.5$^b$ | 3.5±0.7$^a$ | 2.0±0.3$^b$ |
| | EBC | 1.1±0.2$^a$ | 0.9±0.3$^a$ | 2.2±0.9$^a$ | 0.2±0.1$^b$ | 1.7±0.2$^a$ | 0.0±0.0$^b$ |
| IERM | TPC | 3.6±1.3$^a$ | 2.3±0.8$^b$ | 3.8±1.1$^a$ | 3.2±1.2$^b$ | 3.3±0.9$^a$ | 3.3±0.5$^a$ |
| | EBC | <1.0 | 1.0±0.3 | <1.0 | 0.0±0.0 | <1.0 | 1.4±0.0 |

[ab]Mean value in the same row with different letters are significantly (p < 0.05) different

TPC: total plate count, EBC: *Enterobacteriaceae* count, IERM: individual ewe raw milk, US: udder surface SD: standard deviation

**Table 6** Correlation between microbiological parameters

| | TPC$^{IERM}$ | TPC$^{US}$ | EBC$^{IERM}$ | EBC$^{US}$ |
|---|---|---|---|---|
| TPC$^{IERM}$ | 1 | 0.18 | 0.29[*] | 0.02 |
| TPC$^{US}$ | 0.18 | 1 | 0.27[*] | 0.32[**] |
| EBC$^{IERM}$ | 0.29[*] | 0.27[*] | 1 | 0.56[**] |
| EBC$^{US}$ | 0.02 | 0.32[**] | 0.56[**] | 1 |

[*]Correlation is significant at the 0.05 level; [**]Correlation is significant at the 0.01 level

TPC: total plate count, EBC: *Enterobacteriaceae* count, [IERM]individual ewe raw milk, [US]udder surface