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Association of Breed of Sheep or Goats with Somatic Cell Counts and Total Bacterial Counts of Bulk-Tank Milk

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Abstract: The objective was to describe potential associations of somatic cell counts (SCC) and total bacterial counts (TBC) in bulk-tank milk from sheep and goat farms with breeds of these animals in Greece. In total, 325 dairy sheep flocks and 119 dairy goat herds were visited for the collection of milk; the breed of animals in farms was evaluated for a potential association with SCC or TBC. The most frequently seen sheep breeds were the Lacaune (95 flocks) and the Chios (44 flocks). The most frequently seen goat breeds were the indigenous Greek (*Capra prisca*) (50 herds) and the Murciano-Granadina (13 herds). In a multivariable analysis, the breed and the application of machine-milking in sheep flocks, and the breed and the management system in goat herds emerged as significant factors for increased SCC (>0.75×10^6 cells mL^{-1}) in bulk-tank milk. Further, the month of lactation at sampling in sheep flocks emerged a significant factor for increased TBC (>1500×10^3 cfu mL^{-1}) in bulk-tank milk.

Keywords: Chios; genetic improvement; Lacaune; mastitis; mastitis resistance; milk production; *Capra prisca*; somatic cell counts

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1. Introduction

There are over 6,000,000 sheep and 3,000,000 goats in Greece. The predominant type of production is dairy, with over 95% of animals in the country being milked. Many reports are available, presenting morphological characteristics of sheep and goat breeds in Greece (e.g., for sheep: [1–5], for goats: [6,7]).

A genetic background to mastitis in small ruminants has been identified [8–11]. Indeed, in the study of Davies et al. [12], mastitis was considered the top ranking sheep disease amenable for genetic studies. An assessment of mastitis resistance in genetic studies is usually performed by using somatic cell counts in milk; increased somatic cell counts can be employed as an indicator of infection and therefore of subclinical mastitis [13]. Depending on the breed, the heritabilities for somatic cell counts range from 0.09 to 0.30, whilst those for production traits vary between 0.30 and 0.60 [13].

In healthy mammary glands of ewes and does, there are epithelial cells and leucocytes, which predominate. The epithelial cells originate from the mammary gland itself, whilst the origin of leucocytes is the blood. Leucocyte types in the milk of healthy animals include macrophages (40–85%), neutrophils (5–35%) and lymphocytes (10–20%) [14–16]. Leucocytes are part of the innate defences of the mammary gland and play a significant protective role against invading bacteria. Macrophages are the first among the leucocytes present in the mammary gland to counteract the invading pathogens and to initiate the defensive leucocytic response [17]. As part of the defence response of the animal during infection, leucocytes (initially neutrophils) enter into the infected mammary gland, which leads to an increase in the numbers of cell counts in the milk. Therefore, an increased number of somatic cells in the milk of individual animals reflects the presence of an inflammatory process in the mammary gland, i.e., mastitis. With regard to the bulk-tank milk, somatic cell counts are employed to indicate the existence of mastitis in the animals of the farm. In sheep, according to Berthelot et al. [18], somatic cell counts in the bulk-tank milk indicate a mastitis prevalence of 15% in the flock. In the European Union, the current legislation does not provide an officially acceptable threshold for somatic cell counts in the raw milk of sheep and goats. In the United States of America, there is relevant legislation only for goat milk [19], allowing up to 0.75×10^6 cells mL^{-1} in the bulk-tank milk.

Total bacterial counts in the bulk-tank milk express the amount of microbial contamination therein. Bacteria in bulk-tank milk can originate from within the mammary gland, from the udder and teat skin, and from the surface of equipment for milk handling and storage. Hence, total bacterial counts reflect the possible presence of mastitis, udder cleaning procedures during the milking routine and environmental contamination in the farm environment (particularly in the milking parlour). The European Union legislation has set a threshold for total bacterial counts in the bulk-tank milk of small ruminants equal to 1,500,000 colony-forming units (cfu) mL^{-1} for milk that would undergo thermal processing and 500,000 cfu mL^{-1} for milk that would be used for direct consumption [20].

The objective of the work presented herein is to describe the potential associations of somatic cell counts (SCC) and total bacterial counts (TBC) in the bulk-tank milk from sheep and goat farms with breeds of these animals in Greece, with special reference to the potential role of animal breeds.

2. Materials and Methods

2.1. Sheep and Goat Farms, Sampling and Laboratory Examinations

An extensive cross-sectional study was performed. In total, 325 dairy sheep flocks and 119 dairy goat herds in all 13 administrative regions of Greece (Figure 1) were included into the study and visited for the collection of samples and information. Further details regarding these farms are presented in Table S1. Farms were selected by collaborating veterinarians on a convenience basis (willingness of farmers to accept a visit by university personnel for an interview and sample collection). The principal investigators (D.T.L. and G.C.F.) visited all of the farms in the study for sample collection.

During each visit, we collected four 20 mL samples from the bulk-tank milk using appropriate techniques for sampling (two samples were for cell counting, and two samples were for bacterial counting). Then, we stored these samples at 0.0 to 4.0 °C using ice packs in portable refrigerators. We performed somatic cell counting on each of the two samples within 4 h after collection, and we used the remaining two samples for bacterial counting, which was performed within 24 h after the collection of samples. At this stage, two sub-samples were created and processed from each of the four samples, so that we performed each separate test four times (each one in different sub-samples). The transportation of samples to the laboratory was by the investigators and by car; the samples collected from farms in the islands were also transported as ice-packed accompanying luggage by airplane or boat.

The somatic cell counts (SCC) (Lactoscan SCC; Milkotronic Ltd., Nova Zagora, Bulgaria) were measured on each of the four relevant sub-samples. For total bacterial counts (TBC) in the milk samples, the procedures detailed by Laird et al. [21] were followed and the total bacterial count in the initial sample was calculated. After the completion of sample processing for TBC, the temperature of the respective samples was measured and in no case was found to exceed 3.8 °C.

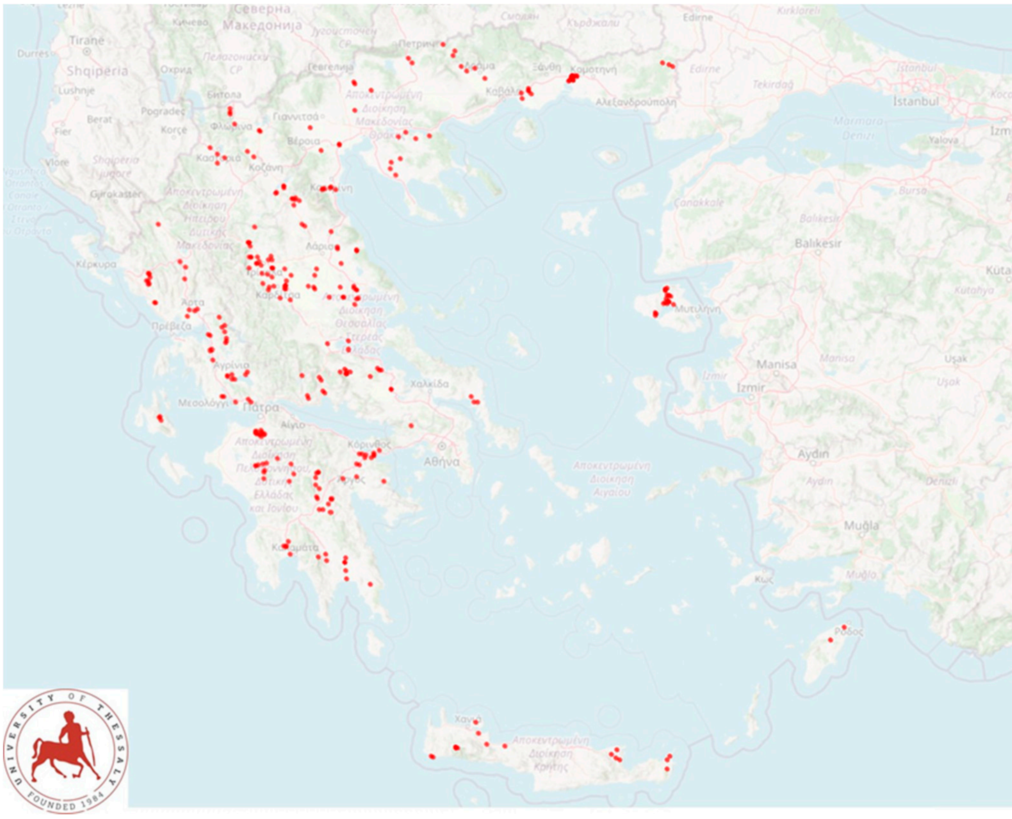

Figure 1. Locations of 444 small ruminant farms around Greece that were visited for bulk-tank milk sampling.

2.2. Data Management and Analysis

During cell counting and bacterial counting, for each bulk-tank milk sample, the results of the two sub-samples from each sample were averaged and, then, the two means were again averaged for the final result regarding each bulk-tank milk.

The data were entered into Microsoft Excel and analysed using SPSS v. 21 (IBM Analytics, Armonk, NY, USA). A basic descriptive analysis was performed. Exact binomial confidence intervals (CI) were obtained. For all statistical analyses and according to the standards employed for analyses of SCC, these were transformed to somatic cell scores (SCS) by using the equation $SCS = \log_2(SCC/100) + 3$ [22,23]. TBC were transformed to $\log_{10}$, and the transformed data were used in the analyses. At the end, for presentation of the results, the transformed findings were back-transformed into $100 \times 2^{(SCS-3)}$ and $10^{\log}$ data.

Initially, SCC and TBC were compared between farms in accordance with the breed of the animals therein, by using an analysis of variance.

Then, the outcomes for "increased SCC in bulk-tank milk" (i.e., with SCC over 0.75×10^6 cells mL^{-1}) and "increased TBC in bulk-tank milk" (i.e., with TBC over 1500×10^3 cfu mL^{-1}) were studied. The following variables were assessed for the potential association with SCC or TBC in the bulk-tank milk of the farms: breed, management system applied in the farm, application of machine-milking in the farm, month of lactation period at sampling, number of animals in the farms, average per animal yearly milk production in the farm and application of teat disinfection at milking. For each of these variables, categories were created. Exact binomial confidence intervals were obtained. Initially, the importance of predictors was assessed using cross-tabulation with Pearson's chi-square test univariable analysis without random effects. Subsequently, multivariable models were created using mixed-effects logistic regression with farms as the random effect, as defined by $\hat{Y} = b_0 + b_1X_1 + b_2X_2 + \ldots + b_iX_i$ (Y: dependent variable, X: independent variables) and initially offering to the model all variables, which achieved a significance of $p < 0.2$ in the univariable analysis. The variables were removed from the initial model by backwards elimination. The p value of removal of a variable was assessed by the likelihood ratio test, and for those with a p value of >0.2, the variable with the largest probability was removed. This process was repeated until no variable could be removed with a p value of >0.2. The variables required for the final multivariable model on each occasion are shown in Table S2.

Separate analyses were performed for sheep flocks and goat herds. Initially, the analyses for the outcomes "increased SCC in bulk-tank milk" and "increased TBC in bulk-tank milk" were performed by taking into account all breeds, independent of the number of farms with each breed. Then, the analyses were repeated by taking into account only farms with the six most frequently recorded sheep breeds and the four most frequently recorded goat breeds.

For the evaluation of the zootechnical characteristics, Pearson's chi-square test or analysis of variance was used as appropriate.

In all analyses, statistical significance was defined at $p < 0.05$.

3. Results

In sheep farms, the breed most frequently seen was the Lacaune breed (95 flocks), followed by the Chios breed (44 flocks). In goat farms, the breed most frequently seen was the indigenous Greek breed (*Capra prisca*) (50 herds), followed by the Murciano-Granadina breed (13 herds). With regard to sheep, breeds of Greek origin (including indigenous and crossbred animals) were present in 186 flocks (57.2%) and imported breeds were present in 139 flocks (42.8%). With regard to goats, breeds of Greek origin (including indigenous and crossbred animals) were present in 74 herds (62.2%) and imported breeds were present in 45 herds (37.8%). The various breeds of sheep and goats present in the farms into the study are shown in Table 1; their zootechnical characteristics are summarized according to the origin of the breeds (i.e., Greek or imported) in Table 2.

Table 1. Geometric mean somatic cell counts and total bacterial counts in bulk-tank milk in small ruminant farms in Greece according to animal breed in the farms.

Breed	Farms (n)	Geometric Mean (95% CI [1])	
		Somatic Cells Counts (Cells mL^{-1})	Total Bacterial Counts (cfu mL^{-1})
		Sheep farms	
Assaf	30	0.528×10^6 (0.427×10^6–0.652×10^6)	329×10^3 (191×10^3–562×10^3)
Awassi	1	0.227×10^6	495×10^3
Boutsko	2	0.374×10^6 (0.333×10^6—0.418×10^6)	425×10^3 (93×10^3—1905×10^3)
Chios	44	0.592×10^6 (0.467×10^6—0.754×10^6)	442×10^3 (185×10^3—977×10^3)
Crossbreds	43	0.547×10^6 (0.439×10^6—0.679×10^6)	547×10^3 (288×10^3—776×10^3)
Friesarta	12	0.457×10^6 (0.296×10^6—0.708×10^6)	461×10^3 (204×10^3—1047×10^3)
Friesian	13	0.530×10^6 (0.421×10^6—0.665×10^6)	216×10^3 (135×10^3—347×10^3)
Karagouniko	5	0.642×10^6 (0.252×10^6—1.638×10^6)	272×10^3 (50×10^3—1479×10^3)
Kefallinia	1	0.172×10^6	677×10^3
Lacaune	95	0.453×10^6 (0.398×10^6—0.515×10^6)	360×10^3 (240×10^3—550×10^3)
'Local' [2]	55	0.516×10^6 (0.412×10^6—0.643×10^6)	496×10^3 (389×10^3—891×10^3)
Mytilini	18	$0.276 \times 1\ 0^6$ (0.192×10^6—0.396×10^6)	408×10^3 (209×10^3—794×10^3)
Sfakia	6	$0.597 \times 1\ 0^6$ (0.412×10^6—0.866×10^6)	357×10^3 (257×10^3—490×10^3)
		Goat farms	
Alpine	9	0.647×10^6 (0.477×10^6—0.878×10^6)	659×10^3 (309×10^3—1413×10^3)
Crossbreds	18	0.799×10^6 (0.579×10^6—1.104×10^6)	305×10^3 (123×10^3—759×10^3)
Damascus	18	0.936×10^6 (0.775×10^6—1.135×10^6)	1009×10^3 (550×10^3—1862×10^3)
Kefallinia	1	1.831×10^6	7482×10^3
Indigenous Greek (*Capra prisca*)	50	0.932×10^6 (0.802×10^6—1.081×10^6)	589×10^3 (437×10^3—871×10^3)
Murciano-Granadina	13	0.657×10^6 (0.471×10^6—0.915×10^6)	608×10^3 (309×10^3—1202×10^3)
Saanen	5	0.653×10^6 (0.424×10^6—1.009×10^6)	281×10^3 (100×10^3—794×10^3)
Skopelos	5	0.766×10^6 (0.415×10^6—1.407×10^6)	625×10^3 (219×10^3—1950×10^3)

[1] CI: confidence interval; [2] the term used by the respective farmers to refer to a variety of small-scale breeds, not always related between them, and each one prevailing only in some areas of the country with limited geographic dissemination.

Table 2. Zootechnical characteristics of 444 small ruminant farms in Greece, summarized according to the origin of the breeds of animals therein.

Origin of Breed [1]	Farms (n)	Management System Applied in the Farms				Application of Machine-Milking		Average No. of Animals per Farm [4]	Average Yearly Milk Production per Animal (L) [4]
		I [2]	s-I	s-E	E	Y [3]	N		
					Sheep farms				
Greek	186	18	69	76	23	128	58	305 ± 16	181 ± 6
Imported	139	25	62	31	1	127	12	247 ± 11	243 ± 8
					Goat farms				
Greek	74	2	9	44	19	35	39	234 ± 22	155 ± 9
Imported	45	7	20	17	1	31	14	244 ± 42	276 ± 20

[1] Greek: indigenous (i.e., breed native of Greece or created in the country) or crossbred (i.e., cross between imported or indigenous breeds), imported: breed brought into Greece from another country; [2] management system applied in farms classified according to the standards of the European Food Safety Authority [24]: I: intensive, s-I: semi-intensive, s-E: semi-extensive, E: extensive; [3] Y: yes, N: no; [4] mean ± standard error of the mean.

In farms with breeds of Greek origin, for sheep and goats, semi-extensive management was applied more frequently (40.9% and 59.5% of farms, respectively). In contrast, in farms with imported breeds, semi-intensive management was applied more frequently (44.6% and 44.4% of farms, respectively) ($p < 0.0001$). In the former farms, average yearly milk production was significantly lower than in the latter: 181 ± 6 L (mean ± standard error of the mean) and 155 ± 9 L per animal for sheep and goat farms, respectively, versus 243 ± 8 and 276 ± 20 L per animal, respectively ($p < 0.0001$). There was a significant progressive reduction in average yearly milk production from intensive to extensive for both sheep ($p < 0.0001$) and goats ($p = 0.0002$) (Figure 2).

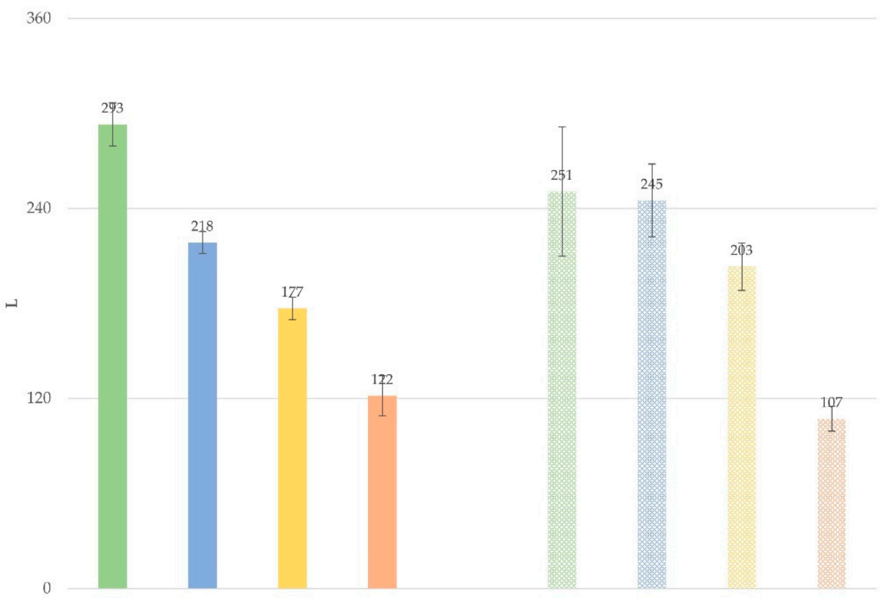

Figure 2. Average yearly milk production per animal in sheep (solid pattern) or goat (motif pattern) farms in Greece, applying the intensive (green), semi-intensive (blue), semi-extensive (yellow) or extensive (red) management system.

3.1. Effect of Breed in Sheep Flocks

The geometric mean SCC and TBC according to animal breed in the sheep flocks of the study are shown in Table 1.

3.1.1. Effect on Somatic Cell Counts

When flocks with all breeds were considered, no significant difference was seen in SCC between the breeds ($p = 0.22$). However, when only flocks with the six most frequently recorded breeds were taken into account, a clear difference in SCC was seen between the breeds ($p = 0.004$) (Table 1). There was no significant difference in SCC between flocks with breeds of Greek origin or imported ones (0.501×10^6 versus 0.473×10^6 cells mL^{-1}, respectively; $p = 0.24$).

For increased SCC ($>0.75 \times 10^6$ cells mL^{-1}) in bulk-tank milk, when flocks with all breeds were considered, a significant association was seen during the univariable analysis only for the application of machine-milking in the farms ($p = 0.001$). When only flocks with the six most frequently recorded breeds were taken into account, a significant association was seen during the univariable analysis for the application of machine-milking in the farms ($p = 0.006$) and the number of animals in the flock ($p = 0.033$) (Table S3). Among the variables included in the multivariable analysis (Table S2), the following emerged as significant factors for increased SCC in the flocks in both analyses: the breed ($p = 0.025$ and 0.014, respectively) (Figure 3) and the application of machine-milking in the farms ($p = 0.0004$ and 0.003, respectively) (Table 3).

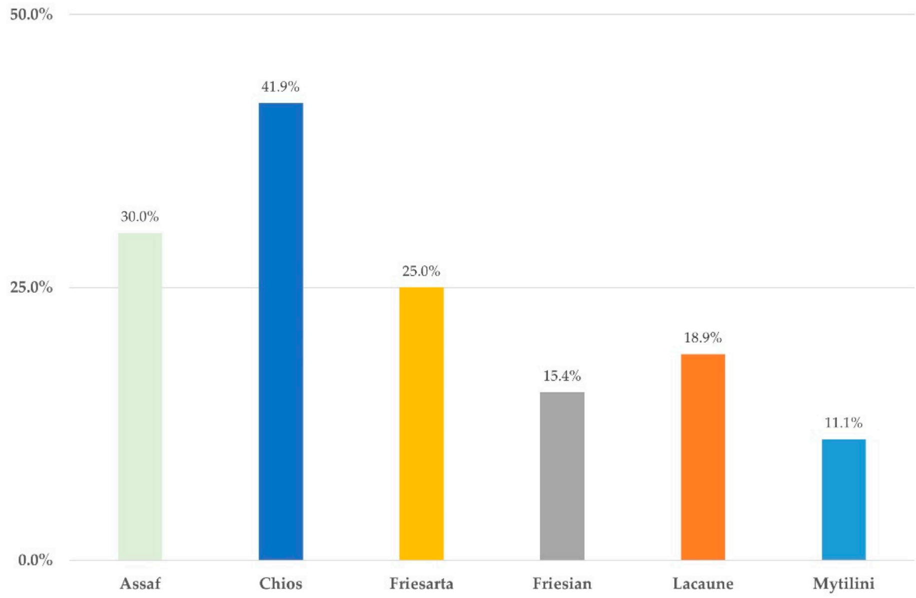

Figure 3. Proportion of flocks with increased somatic cell counts ($>0.75 \times 10^6$ cells mL^{-1}) in the bulk-tank milk of sheep flocks in Greece in accordance with the breed of sheep therein.

Table 3. Results of multivariable analysis for increased somatic cell counts ($>0.75 \times 10^6$ cells mL^{-1}) in the bulk-tank milk of sheep flocks in Greece (mixed effects logistic regression and analysis with only flocks with the six most frequently recorded breeds).

Variables (n = 2)	Odds Ratio [1] (95% Confidence Intervals)	p
Breed		0.014
Assaf (n = 30)	3.429 (0.649–18.114)	0.15
Chios (n = 44)	5.539 (1.132–27.108)	0.035
Friesarta (n = 12)	2.667 (0.373–19.061)	0.33
Friesian (n = 13)	1.455 (0.177–11.937)	0.73
Lacaune (n = 95)	1.870 (0.394–8.873)	0.43
Mytilini (n = 18)	reference	
Application of machine-milking		0.003
Yes (n = 186)	reference	
No (n = 26)	3.129 (1.342–7.296)	0.008

[1] Odds ratios calculated against the lowest prevalence associations of the variables.

3.1.2. Effect on Total Bacterial Counts

No significant difference was seen in TBC between the breeds, either when flocks with all breeds were considered or when only flocks with the six most frequently recorded breeds were taken into account ($p > 0.78$) (Table 1). There was no significant difference in TBC between flocks with breeds of Greek origin or imported ones (451×10^3 versus 336×10^3 cfu mL^{-1}, respectively; $p = 0.11$).

For increased TBC ($>1500 \times 10^3$ cfu mL^{-1}) in bulk-tank milk, when flocks with all breeds were considered, a significant association was seen during the univariable analysis for the application of machine-milking in the farms ($p = 0.032$) and the month of lactation at sampling ($p = 0.006$). When only flocks with the six most frequently recorded breeds were taken into account, a significant association was seen during the univariable analysis for the management system applied in flocks ($p = 0.045$) (Table S4). Among the variables included in the multivariable analysis (Table S2), the following emerged as significant factors for increased TBC in the flocks: the month of lactation at sampling for both analyses ($p = 0.0008$ and 0.014, respectively) and the average per animal yearly milk production in the farm ($p = 0.037$, only when all breeds were considered (Table 4).

Table 4. Results of the multivariable analysis for increased total bacterial ($>1500 \times 10^3$ cells mL^{-1}) in the bulk-tank milk of sheep flocks in Greece (mixed effects logistic regression and analysis with only flocks with the six most frequently recorded breeds).

Variable (n = 1)	**Odds Ratio [1] (95% Confidence Intervals)**	***p***
Month of lactation period at sampling		0.014
Up to 3rd month (n = 64)	2.644 (1.067–6.552)	0.036
3rd to 6th month (n = 54)	2.698 (1.054–6.908)	0.039
After 6th month (n = 94)	reference	

[1] Odds ratios calculated against the lowest prevalence associations of the variables.

3.2. *Effect of Breed in Goat Herds*

The geometric mean SCC and TBC according to animal breed in the goat herds of the study are shown in Table 1.

3.2.1. Effect on Somatic Cell Counts

When herds with all breeds were considered, no significant difference was seen in SCC between the breeds ($p = 0.23$). Similar findings were seen when only herds with the four most frequently recorded breeds were taken into account ($p = 0.48$) (Table 1). There was no significant difference in SCC between herds with breeds of Greek origin or imported ones (0.894×10^6 versus 0.754×10^6 cells mL^{-1}, respectively; $p = 0.11$).

For increased SCC ($>0.75 \times 10^6$ cells mL^{-1}) in bulk-tank milk, in both analyses (i.e., with herds with all breeds and with herds with the four most frequently recorded breeds), a significant association was seen during the univariable analysis only for the breed ($p = 0.012$ and 0.001, respectively) (Table S5). In the multivariable analysis (Table S2), the breed emerged as a significant factor for increased SCC in the herds in both analyses ($p = 0.012$ and 0.042, respectively) (Figure 4), whilst the management system was found to be significant in the analysis with herds with the four most frequently recorded breeds ($p = 0.01$) (Table 5).

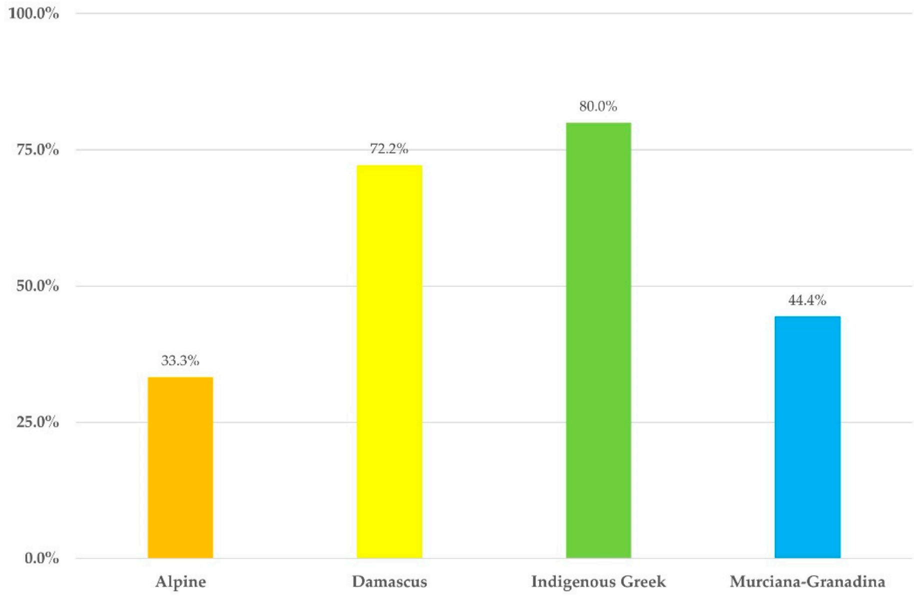

Figure 4. Proportion of herds with increased somatic cell counts (>0.75 $\times$ 10^6 cells mL^{-1}) in the bulk-tank milk of goat herds in Greece in accordance with the breed of goats therein.

Table 5. Results of multivariable analysis for increased somatic cell counts (>0.75 $\times$ 10^6 cells mL^{-1}) in the bulk-tank milk of goat herds in Greece (mixed effects logistic regression and analysis with only herds with the four most frequently recorded breeds).

Variables (*n* = 2)	Odds Ratio [1] (95% Confidence Intervals)	*p*
Breed		0.042
Alpine (*n* = 9)	1.125 (0.183–6.935)	0.90
Damascus (*n* = 18)	5.850 (1.222–27.995)	0.027
Indigenous Greek (*Capra prisca*) (*n* = 50)	9.000 (2.295–35.296)	0.002
Murciana-Granadina (*n* = 18)	reference	
Management system		0.01
Intensive (*n* = 7)	reference	
Semi-intensive (*n* = 22)	3.611 (0.570–22.898)	0.17
Semi-extensive (*n* = 43)	5.769 (0.988–33.677)	0.05
Extensive (*n* = 18)	12.500 (1.600–97.650)	0.016

[1] Odds ratios calculated against the lowest prevalence associations of the variables.

3.2.2. Effect on Total Bacterial Counts

No significant difference was seen in TBC between the breeds, either when flocks with all breeds were considered or when only flocks with the six most frequently recorded breeds were taken into account ($p > 0.23$) (Table 1). There was no significant difference in TBC between herds with breeds of Greek origin or imported ones (522 $\times$ 10^3 versus 694 $\times$ 10^3 cfu mL^{-1}, respectively; $p = 0.29$).

For increased TBC (>1500 $\times$ 10^3 cfu mL^{-1}) in bulk-tank milk, no significant association was seen during the univariable analysis with any variable in both analyses ($p > 0.06$) (Table S5). Among the variables included in the multivariable analysis (Table S2), none emerged as a significant factor for increased TBC ($p > 0.06$).

4. Discussion

In this work, we studied the sheep and goat breeds present in small ruminant farms in Greece and we evaluated possible differences between the breeds in SCC and TBC. Farms from all regions of the country were included into the study; hence, conditions prevailing throughout Greece had been taken into account and regional factors had a smaller influence.

Additionally, during the study, we employed consistent methodologies for sampling and sample processing, whilst the same investigators performed specific tasks and always maintained consistency, which all helped to minimize possible bias. Sheep and goat milk are important agricultural products in Greece, and the country produces approximately 25% of the total European small ruminant milk. However, the possible association of the sheep and goat breeds prevalent in the country in the quality of milk have not been hitherto described.

A significant proportion of the breeds in the farms was imported breeds. This indicates the continuous efforts of farmers to improve the genetic material in their flocks/herds and to achieve better production, with special reference to milk production. It is noteworthy that all imported breeds are characterized for high milk yield. This was reflected in the higher average milk production achieved in those farms than in farms with breeds of Greek origin. Consequently, this could also be seen in the management system applied in the farms; higher milk production requires increased input into farms: infrastructures (e.g., installation of milking systems), reduced grazing, increased staff numbers, etc., all of which are in line with the intensive or semi-intensive management system.

For SCC, as the current EU legislation does not provide a legal threshold, we employed the threshold of 0.75×10^6 cells mL^{-1}, following the relevant US regulations and taking into account that dairy factories in the country use this threshold to regulate payments to farmers according to the quality of milk delivered. A similar practice is also applied in Spain, where payments for milk by dairy factories similarly depend upon the SCC therein [25].

For sheep, genomic selection has been shown to have good accuracy for mastitis resistance [26] but only when predicting resistance in closely related animals [27]. In the present study, it was found that breed was of significance for increased SCC, i.e., above the threshold of 0.75×10^6 cells mL^{-1}. In Lacaune-breed sheep, a long-standing program for improvements in the resistance to mastitis has been running for over 15 years now [8,28]. The development of genomic methods and tools for applications to sheep and the sequencing of the sheep genome [29] have greatly contributed in those efforts [13,30]. In a recent study, Oget et al. [31] have confirmed the QTL regions associated with mastitis resistance in sheep of that breed.

The Greek breed most frequently recorded in the farms was the Chios. These animals are considered highly susceptible to mastitis. For example, Kiossis et al. [32] have reported that *Staphylococcus chromogenes* can survive in the mammary glands of Chios-breed ewes during the dry-period and cause subclinical infection after the subsequent lambing; in another study, Vasileiou et al. [33] have reported that the prevalence of subclinical mastitis in flocks with Chios-breed animals was 32%, higher than the national average of 26% [33]. Although genomic regions associated with specific mastitis traits in Chios-breed sheep have been identified [34], unfortunately, in Greece, no genetic improvement programmes have been developed for resistance to mastitis despite the significance of the breed for the sheep population of the country. This was reflected in the fact that there was a high proportion of farms with Chios-breed animals that had increased somatic cell counts in the bulk-tank milk; in fact, the results indicated that farms with Chios-breed animals had higher odds for increased SCC. Possible reasons for the increased susceptibility of the breed could be the bad udder conformation in animals of this breed, which adversely affects correct milking and can predispose animals to mammary infections [2]. In Latxa- and Sarda-breed animals, a positive association was found between SCC and udder conformation [35,36], suggesting that udders with what is perceived to be a good shape would be less affected by subclinical mastitis. Moreover, in Chios-breed animals, a peri-parturient immunosuppression associated with macrophage and neutrophil function has been found and can contribute to the development of mastitis [37].

In contrast, there was a low proportion of farms with animals of the Mytilini-breed that had increased SCC in bulk-tank milk. Future studies could be based on breed as a potential model for mastitis resistance among Greek breeds. Unfortunately, however, the breed is native to the island of Lesvos, where bluetongue is endemic [38] and, consequently, the Ministry of Agriculture has imposed a total ban on animal movements outside that island.

In goats, there is significantly less work regarding potential resistance to mastitis. Some work has been carried out in Alpine-breed [11] or Saanen-breed [39] animals. Again, in the Greek breeds, no efforts toward relevant genetic improvement have been made and this was reflected in farms with the local Indigenous Greek breed (*Capra prisca*) being at higher odds of having increased SCC in bulk-tank milk. However, no systematic efforts have been carried out as in sheep for resistance to the infection.

However, apart from breed, other factors were also found to be important for increased SCC, specifically the use of hand-milking for sheep and the application of semi-extensive or extensive management systems for goats (in which most often hand-milking is used). Similar findings have been reported in Spain by Gonzalo et al. [40], who studied four breeds (Assaf, Awassi, Churra and Castelana) and found that SCC in hand-milked flocks were higher than in machine-milked ones. In hand-milked ewes, staphylococci from the hands of milkers can be transmitted to the teats of ewes [41]. Likely as a consequence of that, hand-milking has been found to result in increased bacterial colonisation within the teat duct [42]. These bacteria can lead to mastitis, when the local defences of the teat are impaired [43,44], during which SCC increases, and reflect higher SCC in the bulk-tank milk of the farm.

In contrast, in goats, no such differences were reported in a study of two breeds (Alpine and Nubian) and SCC did not differ between machine- or hand-milked herds [45]. In goat farms, we found that the application of extensive or semi-extensive management systems can be significant factors for increased SCC. In these conditions, indeed hand-milking is more frequently practiced and certainly this may be a contributing factor, but not to as large an extent as in sheep [45]. Other factors can also play a role in these conditions; for example, incorrect nutrition can lead in increased incidence of mastitis [46] causing increased SCC, but on the other hand, it should be noted that the reduced milk production found in herds under extensive or semi-extensive management can also be a partial determinant for the increased SCC [47].

With regard to TBC, no effect of breed was evident. This was reasonable given that total bacterial counts depend on sources other than the mammary gland. TBC can reflect bacterial populations on the udder skin (transferred to milk at the time of milking) as well as from the surface of milk handling and storage equipment, e.g., teatcups, pipelines of the milking parlour and milk tanks, or from the hands of milkers themselves in flocks/herds where hand-milking is applied. Although there is a correlation between SCC and TBC in milk, the role of animal breed was not found to be important for TBC in bulk-tank milk.

5. Conclusions

In an extensive countrywide study throughout Greece, it was found that the animal breed had an effect on the presence of increased somatic cell counts (>0.75×10^6 cells mL^{-1}) in the bulk-tank milk of sheep and goat farms.

The above also underlines the importance of introducing mastitis resistance as a trait for potential genetic improvement in the sheep and goat industry in Greece. In a country where sheep and goats constitute a large part of the agriculture sector and which has a large proportion of milk production in Europe [48], genetic improvement for mastitis resistance will contribute to better health and welfare of sheep and goats and to increased productivity on farms.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11167356/s1, Table S1: Zootechnical characteristics of 444 small ruminant farms in Greece, classified in accordance with the breed of animals therein. Table S2: Details of multivariable models employed for the evaluation of "increased somatic cell counts in bulk-tank milk" (i.e., with SCC above 0.75×10^6 cells mL^{-1}) and "increased total bacterial counts in bulk-tank milk" (i.e., with TBC above 1500×10^3 cfu mL^{-1}) with the zootechnical characteristics of 444 small ruminant farms in Greece. Table S3: Associations of zootechnical characteristics with increased somatic cell counts in bulk-tank milk (i.e., above 0.75 × 10^6 cells mL^{-1}) in 325 sheep farms in Greece, as found in a univariable analysis. Table S4: Associations of zootechnical characteristics with increased total bacterial counts in bulk-tank milk (i.e., above 1500 × 10^3 cfu mL^{-1}) in 325 sheep farms in Greece, as found in a univariable analysis. Table S5: Associations of zootechnical characteristics with increased somatic cell counts in bulk-tank milk (i.e., above 0.75 × 10^6 cells mL^{-1}) in 119 goat farms in Greece, as found in a univariable analysis. Table S6: Associations of zootechnical characteristics with increased total bacterial counts in bulk-tank milk (i.e., above 1500 × 10^3 cfu mL^{-1}) in 119 goat farms in Greece, as found in a univariable analysis.

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