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Altitude-related variations in microbiological quality of raw sheep milk and Vlašić cheese

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Summary

This study investigated the effect of altitude on the microbiological quality of raw sheep milk and Vlašić cheese produced at three locations (Galica, Gostilj, and Paklarevo) on Mount Vlašić, Bosnia and Herzegovina. A total of 30 samples were analysed for the presence of *Salmonella* spp. and *Listeria monocytogenes*, and for the quantification of coagulase-positive staphylococci, aerobic mesophilic bacteria, *Escherichia coli*, *Enterobacteriaceae*, yeasts, and moulds. None of the samples contained *Salmonella* spp. or *Listeria monocytogenes*. In milk samples, significant differences were found among localities for moulds, with regression analysis indicating a significant negative correlation between altitude and mould count ($p < 0.001$, $R^2 = 0.486$). In Vlašić cheese samples, aerobic mesophilic bacteria were abundant at all sites, while regression analysis showed that *E. coli* ($p < 0.001$, $R^2 = 0.472$) and *Enterobacteriaceae* ($p < 0.001$, $R^2 = 0.767$) counts varied significantly with altitude. No significant correlations with altitude were observed for other microorganisms. The results suggest that differences among localities and altitude-related trends reflect the influence of variations in cheese-making practices on microbial dynamics in artisanal dairy systems. Improving hygiene control, as well as expanding altitude-based studies across different regions, could enhance both product safety and the preservation of traditional cheesemaking practices.

Keywords

sheep milk; Vlašić cheese; foodborne pathogens; microbiological quality; altitude

Sheep milk significantly differs from cow milk due to its higher protein content, viscosity, enhanced nutritional value, and better bactericidal activity in the first hours after milking, which makes it more suitable for processing into cheese [1]. Because the fat globules are smaller in diameter, lipids from sheep milk are easier to digest and more efficiently metabolised compared to cow milk [2]. Cheeses produced from sheep milk have a characteristic taste and aroma, which result from a specific composition of fat and protein [3].

Bosnia and Herzegovina (B&H) has a long tradition of producing indigenous cheeses, the most famous of which is Travnički cheese, also known as Vlašić cheese, which has been produced for over 140 years in the Vlašić mountain area [4]. Milk from Vlašićka Pramenka, an indigenous breed of sheep with high milk potential, is the basis for the production of this cheese [5]. Vlašić cheese belongs to the group of soft, brined cheeses, and the simple technology, based on raw milk and traditional procedures, enables its production in mountain conditions, but also makes it difficult to standardise the quality [3]. Production of this cheese is seasonal, usually starting in May and lasting until early October. During this period, sheep are fed only on the pastures [6]. According to the traditional recipe, the technology of Vlašić cheese production comprises the following steps:

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milk coagulation, curd handling and processing, whey drainage, cutting of the curd mass, layering of the cheese in traditional wooden vats, salting, ripening, and packaging [4, 7]. Its simple production technology enables traditional manufacture under basic mountainous conditions; however, this also poses significant challenges for product standardisation, as substantial variability exists among individual household producers [3]. To date, industrial-scale production of Vlašić cheese does not exist. Unpasteurised milk, used in the production of Vlašić cheese, poses a challenge in terms of hygiene, as microbiological contamination can occur during milking, processing, or storage [7, 8]. Since it is a perishable food, the presence of pathogenic microorganisms in sheep milk and cheeses represents a health risk for consumers and an economic loss for producers [9, 10]. A particular problem of soft cheeses, including Vlašić cheese, is the favourable composition for the growth of pathogenic bacteria such as *Salmonella* spp., *Listeria monocytogenes*, *E. coli*, and coagulase-positive staphylococci [9, 11].

Previous research in B&H indicates a high prevalence of microbiological contaminants in raw sheep milk and Vlašić cheese, most often as a consequence of unhygienic production and processing conditions [6, 12, 13]. Since these products play a significant role in traditional nutrition and local economy, it is important to assess their microbiological status and identify factors that affect safety and quality, including the influence of geographical and production specificities in mountainous areas. Therefore, the objective of this study was to assess the microbiological status of raw sheep milk and Vlašić cheese produced at different altitudes in the mountain Vlašić. The working hypothesis was adopted to explain whether the altitude is associated with measurable variations in the microbiological profiles of these products, reflecting both environmental and hygienic differences among production sites.

MATERIALS AND METHODS

Sampling

The sheep milk sampled in this research comes from a native breed of sheep – Pramenka. The sampling and transport of milk and cheese samples were carried out according to ISO 7218:2024 [14]. Sampling was performed without subsequent contamination, spoilage or damage to the sample.

Firstly, raw sheep milk was sampled, followed by a sampling of Vlašić cheese made from that milk after 24 days of ripening. From each location, 5 milk and 5 cheese samples were taken. A total of 15 milk samples and 15 cheese samples were collected in amounts of approximately 200 ml or 200 g. Samples were collected from three areas in the mountain Vlašić, at different altitudes above sea level (asl): Paklarevo (800 m), Gostilj (900 m), and Galica (1050 m), where Vlašić cheese is traditionally made from raw sheep milk. The samples were transported to the laboratory at a temperature of +4 °C, and were immediately analysed.

Laboratory analysis

All 30 samples were analysed for the presence of *Salmonella* spp. and *Listeria monocytogenes*. Detection and enumeration were performed for the following microorganisms: coagulase-positive staphylococci, aerobic mesophilic bacteria, *Escherichia coli, Enterobacteriaceae*, yeasts and moulds. Analyses were conducted in the Laboratory for Microbiology of Food and Feed of the Veterinary Institute of the Veterinary Faculty (University of Sarajevo, Sarajevo, Bosnia and Herzegovina).

Sample preparation was performed in accordance with ISO 6887-5:2020 [15]. During the sample preparation for analysis, the surface of the container containing the sample at the point where the opening is performed was cleaned with a cotton swab soaked in 96% ethanol to prevent cross-contamination of the sample.

For the detection of *Salmonella* spp. and *Listeria monocytogenes*, 25 g or 25 ml of the sample, or 10 g or 10 ml for the detection and quantification of other microorganisms, was weighed into a sterile Erlenmeyer flask. Before use, buffered peptone water (BPW, Condalab, Madrid, Spain) was tempered to room temperature (21–23 °C), after which the first dilution with BPW was made according to the ratio 9 : 1. Afterwards, the flasks were placed on an orbital shaker (Bibby Scientific, Stone, United Kingdom) for 15 min.

If the microbiological analysis required further dilutions, 1 ml of the initial dilution was transferred to a test tube containing 9 ml of BPW using a sterile pipette or micropipette. After that, the test tube was covered and homogenised using a Vortex mixer (Barnstead International, Dubuque, Iowa, USA) for 5–10 s to obtain a 10^{-2} dilution. Subsequent dilutions were successively prepared in the previously described manner (10^{-3}, 10^{-4}, etc.).

Microbiological analysis of sheep milk and cheese was carried out in accordance with the valid editions of the following ISO standards: ISO 6579-1:2017 [16], ISO 11290-1:2017 [17], ISO 6888-1:2021/Amd 1:2023 [18], ISO 21528-2:2017

[19], ISO 4833-1:2013 [20], ISO 16649-2:2001 [21] and ISO 21527-1:2008 [22].

The original dehydrated media and their additives, reagents and chemicals prescribed by the applied ISO standards were used.

Statistical analysis

Statistical analysis was performed using statistical tools MS Excel (Microsoft, Redmond, Washington, USA) and R Studio (Posit, Boston, Massachusetts, USA).

ANOVA (one-way) analysis of variance was performed to determine if there is a significant difference between the averages of three groups of samples from three locations in Vlašić. If the obtained p-value was less than 0.05, a Tukey post-hoc test was performed to determine whether there was a significant difference between the groups of samples.

A simple linear regression model was used to predict the change in the number of microorganisms as a function of altitude.

RESULTS AND DISCUSSION

The microbiological analysis of raw sheep milk samples (Tab. 1) from Paklarevo, Gostilj, and Galica revealed no presence of *Salmonella* spp. or *Listeria monocytogenes*. Mean counts of aerobic mesophilic bacteria were 2.98×10^5 CFU·ml^{-1} (Galica), 2.51×10^5 CFU·ml^{-1} (Gostilj), and 3.24×10^5 CFU·ml^{-1} (Paklarevo). *E. coli* counts averaged 2.02×10^3 CFU·ml^{-1} (Galica), 0.82×10^3 CFU·ml^{-1} (Gostilj), and 1.73×10^3 CFU·ml^{-1} (Paklarevo). *Enterobacteriaceae* levels were 4.56×10^4 CFU·ml^{-1} (Galica), 3.75×10^4 CFU·ml^{-1} (Gostilj), and 5.59×10^4 CFU·ml^{-1} (Paklarevo). Coagulase-positive staphylococci were detected only in Galica and Gostilj. Yeasts and moulds were present in all samples. Significant differences were observed in *E. coli, Enterobacteriaceae*, and mould levels among localities, and p-values are presented in Tab. 1.

The detection of enterobacteria and coagulase-positive staphylococci in raw milk highlights the influence of milking and handling practices on microbial composition. While the present study focused on food safety indicators and pathogens rather than microbial community profiling, findings from studies on traditional mountain cheeses provide useful context for interpreting altitude- and locality-related differences in microbial counts. Similar findings have been reported for raw milk used in traditional cheeses produced in Alpine [23, 24] and Caucasus regions [25], where microbial loads reflect a combination of on-farm hygiene, environmental exposure, and altitude-related climatic conditions rather than industrial standardisation. These systems are characterised by greater microbial variability, which increases sensitivity to handling practices during milking and storage. Psychrotrophic microorganisms are of particular concern in this context, as they enter milk during milking and storage and produce extracellular enzymes that resist heat treatment, affecting cheese ripening [26, 27].

To determine the correlation between the number of microorganisms in sheep milk samples and altitude, a linear regression analysis was performed. It was calculated to predict the number of microorganisms based on altitude. There was a significant regression equation for mould count in sheep milk (F = 15.13, p < 0.001), with an R^2 of 0.486. The predicted decrease in mould count was 4.12×10^3 CFU·ml^{-1} for each meter of altitude (Fig. 1). The regression statistics, ANOVA, and the coefficients for calculating the correlation are presented in Tab. 2. There was no significant correlation between the number of other microorganisms tested and altitude. Although the direct effects of altitude on specific microbial groups, such as moulds, are understudied, research on traditional dairy systems demonstrates that altitude-related environmental variables, including lower temperatures, increased ultraviolet radiation, and changes in pasture ecology, can influence the composition of fungal and bacterial communities in raw milk and artisanal cheeses. Studies from Alpine raw milk systems and artisanal cheeses along elevation gradients support the notion that microbial assemblages change with altitude, thereby providing eco-

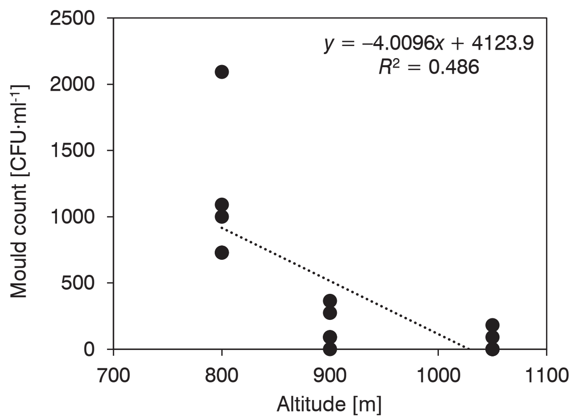

Fig. 1. Linear regression of mould count in sheep milk samples in relation to the altitude.

Tab. 1. Microbiological status of raw sheep milk samples from three locations on Mount Vlašić.

Sample No.	*Salmonella* spp.	Aerobic mesophilic bacteria	*E. coli*	*Enterobacteriaceae*	Yeasts	Moulds	*Listeria monocytogenes*	Coagulase-positive staphylococci
	[CFU·ml^{-1}]	[×10^5 CFU·ml^{-1}]	[×10^3 CFU·ml^{-1}]	[×10^4 CFU·ml^{-1}]	[×10^3 CFU·ml^{-1}]	[CFU·ml^{-1}]	[CFU·ml^{-1}]	[CFU·ml^{-1}]
Galica (1 050 m asl)								
1	ND	5.09	1.36	2.76	9.27	< 10.00	ND	100.00
2	ND	2.55	2.07	4.39	6.09	90.90	ND	9.09
3	ND	2.91	1.47	5.35	7.73	< 10.00	ND	180.00
4	ND	2.40	2.00	5.65	3.73	182.00	ND	< 10.00
5	ND	1.95	3.18	4.66	2.82	< 10.00	ND	< 10.00
Gostilj (900 m asl)								
6	ND	3.04	0.63	5.75	8.55	< 10.00	ND	54.50
7	ND	2.58	1.33	3.56	1.82	364.00	ND	54.50
8	ND	1.38	0.84	2.76	2.27	273.00	ND	9.09
9	ND	2.84	0.54	3.84	7.73	90.90	ND	127.00
10	ND	2.73	0.75	2.84	6.27	90.90	ND	36.40
Paklarevo (800 m asl)								
11	ND	1.98	2.48	5.05	3.27	1 090.00	ND	< 10.00
12	ND	3.05	2.07	5.78	3.45	727.00	ND	< 10.00
13	ND	4.98	2.05	6.22	5.09	2 090.00	ND	< 10.00
14	ND	1.31	1.91	5.15	5.55	1 000.00	ND	< 10.00
15	ND	4.87	0.13	5.75	6.73	727.00	ND	< 10.00
p-value								
	N/A	0.66	0.04 *	0.03 *	0.78	< 0.01 *	N/A	0.07

asl – above sea level, ND – not detected, N/A – not applicable, * – statistically significant difference.

Tab. 2. Regression statistics, ANOVA and coefficients for calculating the correlation of mould count in sheep milk samples in relation to the altitude.

Regression statistics	
Multiple *R*	0.723175
R Square	0.522982
Adjusted *R* Square	0.486289
Standard error	422.6063
Observations	15

ANOVA						
	df	*SS*	*MS*	*F*	Significance *F*	
Regression	2	3 485 399	1 742 700	15.134	0.0005236	
Residual	12	1 381 818	115 152			
Total	14	4 867 217				
	Coefficients	Standard error	*t* Stat	*P*-value	Lower 95%	Upper 95%
Intercept	4 123.923	979.652	4.20958	0.0010212	2 007.513	6 240.334
X variable 1	–4.009569	1.062062	–3.77527	0.0005236	–6.304014	–1.715125

Tab. 3. Microbiological status of Vlašić cheese samples from three locations on Mount Vlašić.

Sample No.	*Salmonella* spp.	Aerobic mesophilic bacteria	*E. coli*	*Enterobacteriaceae*	Yeasts	Moulds	*Listeria monocytogenes*	Coagulase-positive staphylococci
	[CFU·g^{-1}]	[×10^5 CFU·g^{-1}]	[×10^2 CFU·g^{-1}]	[×10^4 CFU·g^{-1}]	[CFU·g^{-1}]	[CFU·g^{-1}]	[CFU·g^{-1}]	[CFU·g^{-1}]
Galica (1 050 m asl)								
1	ND	8.23	24.40	4.18	< 10.00	< 10.00	ND	< 10.00
2	ND	4.69	26.60	3.45	< 10.00	< 10.00	ND	< 10.00
3	ND	2.29	21.80	4.27	< 10.00	< 10.00	ND	< 10.00
4	ND	3.13	18.80	2.73	< 10.00	< 10.00	ND	< 10.00
5	ND	2.40	15.90	3.55	< 10.00	< 10.00	ND	< 10.00
Gostilj (900 m asl)								
6	ND	4.87	1.09	1.18	< 10.00	< 10.00	ND	< 10.00
7	ND	5.12	5.45	1.91	< 10.00	< 10.00	ND	< 10.00
8	ND	4.58	0.91	0.27	< 10.00	< 10.00	ND	< 10.00
9	ND	3.16	0.46	0.18	< 10.00	< 10.00	ND	< 10.00
10	ND	3.70	0.55	0.27	< 10.00	< 10.00	ND	< 10.00
Paklarevo (800 m asl)								
11	ND	2.69	3.27	0.18	< 10.00.	< 10.00	ND	< 10.00
12	ND	1.85	8.36	0.36	< 10.00	< 10.00	ND	< 10.00
13	ND	1.38	1.45	0.63	< 10.00	< 10.00	ND	< 10.00
14	ND	1.82	8.18	0.27	< 10.00	< 10.00	ND	< 10.00
15	ND	1.12	14.50	1.00	< 10.00	< 10.00	ND	< 10.00
p-value								
	N/A	0.04 *	< 0.01 *	< 0.01 *	N/A	N/A	N/A	N/A

asl – above sea level, ND – not detected, N/A – not applicable, * – statistically significant difference.

Tab. 4. Regression statistics, ANOVA and correlation coefficients for *Escherichia coli* count in Vlašić cheese samples in relation to the altitude.

Regression statistics	
Multiple *R*	0.714130
R Square	0.509982
Adjusted *R* Square	0.472289
Standard error	684.5815
Observations	15

ANOVA						
	df	*SS*	*MS*	*F*	Significance *F*	
Regression	2	10 475 052	5 237 526	32.097	0.00001526	
Residual	12	1 958 115	163 176			
Total	14	12 433 200				
	Coefficients	Standard error	*t* Stat	*P*-value	Lower 95%	Upper 95%
Intercept	–4788.756	1586.943	–3.01760	0.00989814	–8217.137	–1360.375
X Variable 1	6.328230	1.720438	3.67827	0.00001526	2.611450	10.045010

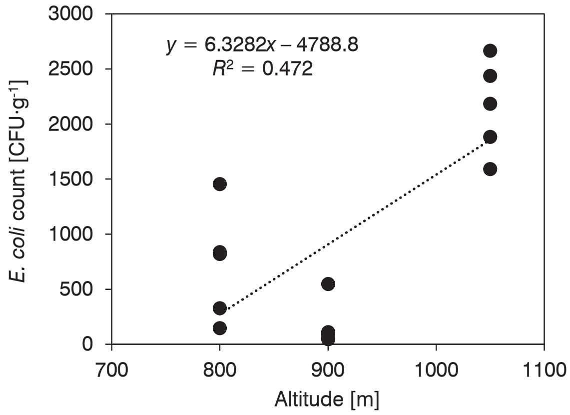

Fig. 2. Linear regression of *Escherichia coli* count in Vlašić cheese samples in relation to the altitude.

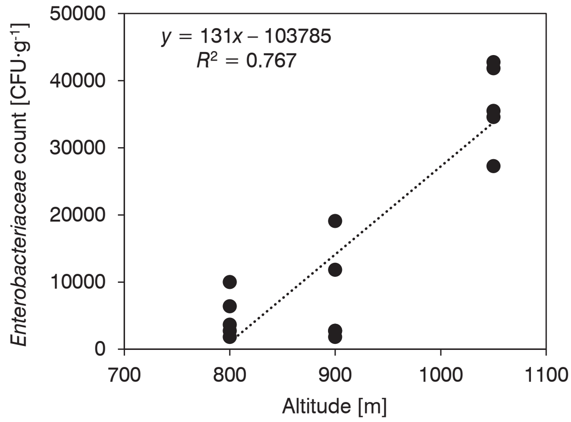

Fig. 3. Linear regression of *Enterobacteriaceae* count in Vlašić cheese samples in relation to the altitude.

logical context for our observed altitude-related trends in indicator organisms [28, 29].

The microbiological analysis of Vlašić cheese samples (Tab. 3) from all three locations tested negative for *Salmonella* spp., *Listeria monocytogenes*, yeasts, moulds, and coagulase-positive staphylococci. Thus, aerobic mesophilic bacteria were abundant: 4.15×10^5 CFU·g^{-1} (Galica), 4.28×10^5 CFU·g^{-1} (Gostilj), and 1.77×10^5 CFU·g^{-1} (Paklarevo). *E. coli* counts were highest in Galica, while *Enterobacteriaceae* levels varied significantly across sites.

A statistically significant difference (with *p*-values given in Tab. 3) between the results from the three different locations was observed for all three groups of microorganisms quantified in the cheese samples. A post-hoc Tukey test determined a statistically significant difference in the number of aerobic mesophilic bacteria between the Vlašić cheese samples from the Gostilj and Paklarevo locations, and for *E. coli* and *Enterobacteriaceae*, a statistically significant difference was determined between the samples from the Gostilj and Galica locations, as well as between the Galica and Paklarevo locations. Cheese samples complied with the microbiological criteria of the Bosnian-Herzegovinian Rulebook on microbiological criteria for food [30], with all results for coagulase-positive staphylococci falling within permissible ranges (10^4–10^5 CFU·g^{-1}). Importantly, yeasts and moulds were not detected, in contrast to previous studies that reported their presence during ripening [6]. This discrepancy underscores the influence of locality and production conditions on microbial outcomes. Studies on artisanal cheeses have demonstrated that yeasts and filamentous fungi typically establish and proliferate during later stages of ripening, contributing to biochemical transformations and flavour development in traditional products. Additionally, yeasts are recognised as key players in surface ripening processes, where their metabolic activities modify pH and enable subsequent microbial succession critical to sensory development [31, 32]. The absence of detectable yeasts and moulds in the present study likely reflects differences in ripening duration, brining intensity, and storage conditions in traditional Vlašić cheese production, rather than an absence of fungal potential in the environment.

A simple linear regression was calculated to predict the number of microorganisms in Vlašić cheese samples based on altitude. A significant regression equation was found ($F = 32.10$, $p < 0.001$), with an R^2 of 0.472 for *E. coli* in Vlašić cheese samples. The predicted increase in the number of *E. coli* is 4.78×10^3 CFU·g^{-1} for each meter of altitude (Fig. 2). Regression statistics, ANOVA and correlation coefficients are shown in Tab. 4. Also, a significant regression equation was calculated ($F = 42.20$, $p < 0.001$), with an R^2 of 0.767 for *Enterobacteriaceae* in Vlašić cheese samples. The predicted increase in the number of *Enterobacteriaceae* is 1.037×10^5 CFU·g^{-1} for each meter above sea level (Fig. 3). Regression statistics, ANOVA and correlation coefficients are presented in Tab. 5. There was no significant correlation between the number of aerobic mesophilic bacteria and altitude ($p < 0.001$).

Vlašić cheese is one of the most prominent elements of the cultural and gastronomic heritage of B&H. Its production remains rooted in traditional household practices, which contribute to its authenticity but also result in variability of quality and potential microbiological risks [3]. Such practices, combined with the absence of milk

Tab. 5. Regression statistics, ANOVA and correlation coefficients for *Enterobacteriaceae* count in Vlašić cheese samples in relation to the altitude.

Regression statistics	
Multiple *R*	0.885116
R Square	0.783430
Adjusted *R* Square	0.766770
Standard error	7601.534
Observations	15

ANOVA					
	df	*SS*	*MS*	*F*	Significance *F*
Regression	2	3 036 804 407	1 518 402 204	42.204	0.00000372
Residual	12	431 735 537	35 977 961		
Total	14	3 468 539 944			

	Coefficients	Standard error	*t* Stat	*P*-value	Lower 95%	Upper 95%
Intercept	−103 784	17 621.274	−5.88974	0.00005322	−141853.137	−65716.241
X Variable 1	131.00478	19.103593	6.85760	0.00000372	89.733981	172.275588

pasteurisation, create conditions that may compromise hygienic standards. Comparisons among the three localities revealed statistically significant differences in several microbial groups. Regression analysis indicated a negative association between altitude and mould counts in milk, but a positive association between altitude and *E. coli* and *Enterobacteriaceae* in cheese. In high-altitude production systems, limited temperature control and greater reliance on ambient environmental conditions may facilitate the survival or introduction of enteric bacteria during cheesemaking. The observed increase in *E. coli* and *Enterobacteriaceae* with altitude in Vlašić cheese is therefore more likely attributable to altitude-associated production constraints than to intrinsic properties of the raw milk. These findings suggest that altitude and related environmental stressors may shape microbial ecology in dairy systems. While previous work has emphasised the role of altitude in biodiversity and microbial adaptation [33], the mechanisms behind the increase of enteric bacteria at higher altitudes remain unclear. Given the adaptability of *E. coli* to a wide range of temperatures and pH values [34], its persistence in brined cheeses is not unexpected, yet it warrants further investigation with larger datasets.

The absence of *Salmonella* spp. and *L. monocytogenes* is reassuring and highlights the protective role of traditional production elements such as brining and low pH. However, the detection of indicator microorganisms such as *E. coli* and *Enterobacteriaceae* underlines the need for stricter hygiene at all stages of production. A recent study [35] investigated the prevalence of key foodborne pathogens – *L. monocytogenes*, *E. coli*, and coagulase-positive staphylococci – in cured raw milk cheeses from the Alentejo region of Portugal. *L. monocytogenes* and coagulase-positive staphylococci exceeding regulatory thresholds were detected in a notable proportion of samples (15.6 % and 16.9 %, respectively), as were pathogenic *E. coli* isolates with antimicrobial resistance profiles, highlighting consumer safety concerns in artisanal cheese systems. In contrast, none of the *Salmonella* spp. or *L. monocytogenes* were detected in raw milk or Vlašić cheese in the present study, although aerobic mesophilic bacteria, *E. coli*, and *Enterobacteriaceae* were consistently present. The absence of major pathogens in our samples may reflect differences in production environments, cheesemaking practices, or ripening conditions between the Portuguese and Bosnian traditional systems. However, both studies underscore that indicator organisms such as *E. coli* and coagulase-positive staphylococci remain useful biomarkers for hygiene status and potential contamination risks in artisanal cheeses made from raw milk.

Although raw sheep milk in the region is rarely consumed as such, consumer safety relies on both traditional protective measures and improved sanitation practices. Some studies have dealt with differences in the composition of cow milk in relation to altitude [36], but studies for sheep milk are scarce, especially when it comes to microbiological variations.

This study is limited by a relatively small sample size, mainly due to producers' hesitation at the time of sampling. In addition, seasonal variation was not considered. Therefore, the results should be interpreted as preliminary. Overall, this study demonstrates that while Vlašić cheese retains microbiological acceptability within current legal standards, altitude and local production practices significantly influence microbial profiles. Future research should integrate larger sample sets and seasonal variation to clarify the ecological drivers of microbial dynamics in mountain dairy systems and to identify interventions that preserve both safety and traditional authenticity.

CONCLUSIONS

This study confirmed that Vlašić cheese generally complies with microbiological safety standards, with *Salmonella* spp., *L. monocytogenes*, yeasts, moulds, and coagulase-positive staphylococci absent in all analysed milk and cheese samples. Although most raw milk samples met regulatory limits, the detection of aerobic mesophilic bacteria, *E. coli*, and *Enterobacteriaceae* in cheese highlights critical points where hygiene during milking, storage, and processing can be improved. Through an innovative framework that incorporates geographical variability and altitude-dependent trends, this study shows that environmental conditions exert a significant impact on microbial dynamics in artisanal dairy production. These findings emphasise the need for stricter hygiene protocols and optimisation of production steps, particularly minimising the interval between milking and cheesemaking, to limit microbial proliferation. Future research should expand sampling across diverse mountainous regions and integrate ecological variables to better understand altitude-associated microbial shifts. Such evidence will contribute to targeted interventions that protect consumer health while preserving the authenticity and cultural value of traditional Vlašić cheese.

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REFERENCES

1. Merlin Junior, I. A. – Santos, J. S. – Costa, L. G. – Costa, R. G. – Ludovico, A. – Rego, F. C. – Santana, E. H.: Sheep milk: physical-chemical characteristics and microbiological quality. ALAN Archivos Latinoamericanos de Nutricion, *65*, 2015, pp. 193–198. ISSN: 0004-0622.
2. Dagdelen, U. – Esenbuga, N.: Effect of breed, age and pasture periods on milk yield, milk components, somatic cell counts and lipid profiles of raw milk from Morkaraman and Tushin sheeps. Large Animal Review, *28*, 2022, pp. 193–198. ISSN: 1124-4593. <https://www.largeanimalreview.com/index.php/lar/article/view/500/192>
3. Hrković, A. – Hodžić, A. – Sarić, Z. – Hamamdžić, M. – Vegara, M. – Šaljić, E. – Juhas-Pašić, E.: Utjecaj kemijskog sastava ovčjeg mlijeka na kemijski sastav Livanjskog i Travničkog sira. (Influence of chemical composition of sheep milk on the chemical composition of Livno and Travnik cheese.) Mljekarstvo, *61*, 2011, pp. 175–181. ISSN: 0026-704X. <https://hrcak.srce.hr/file/103078> In Croatian.
4. Sarić, Z. – Bijeljac, S.: Autohtoni sirevi Bosne i Hercegovine. (Autochthonous cheeses of Bosnia and Herzegovina.). Mljekarstvo, 53, 2003, pp. 135–143. ISSN: 0026-704X. <https://hrcak.srce.hr/file/2696> In Bosnian.
5. Nenadović, K. – Karać, P. – Vučinić, M. – Teodorović, R. – Živanov, D. – Trailović, R. – Beckei, Z. – Janković, L.: Assessment of the welfare of extensively managed autochthonous sheep breed Vlasicka Zackel using animal-based measurements. Acta Veterinaria Belgrade, *70*, 2020, pp. 207–218. DOI: 10.2478/acve-2020-0015.
6. Sakić-Dizdarević, S. – Dizdarević, T. – Kasumović, E. – Sarić, Z. – Mehmeti, I. – Abrahamsen, R. K. – Narvhus, J. A.: Microbiological aspects of the traditional Travnik/Vlašić cheese manufactured in Bosnia and Herzegovina. Journal of Infection in Developing Countries, *17*, 2023, pp. 236–240. DOI: 10.3855/JIDC.17405.
7. Dozet, N. – Adžić, N. – Stanišić, M. – Živić, N.: Autohtoni mliječni proizvodi. (Traditional dairy products.) Podgorica : Poljoprivredni institut, 1996. ISBN: 86-7014-008-X. In Serbian.
8. Kováčová, M. – Výrostková, J. – Dudriková, E. – Zigo, F. – Semjon, B. – Regecová, I.: Assessment of

quality and safety of farm level produced cheeses from sheep and goat milk. Applied Sciences, *11*, 2021, article 3196. DOI: 10.3390/app11073196.
9. Guizani, N. – Kasapis, S. – Al-Attabi, Z. H. – Al-Ruzeiki, M. H.: Microbiological, physicochemical, and biochemical changes during ripening of camembert cheese made of pasteurized cow's milk. International Journal of Food Properties, *5*, 2002, pp. 483–494. DOI: 10.1081/jfp-120015486.
10. Khalaf, A. T.: Detection of bacterial and fungal contamination unsalted and salted cheese in markets of Samara city-Iraq. International Journal of Health Sciences, *6*, 2022, pp. 4611–4620. DOI: 10.53730/ijhs.v6nS3.6918.
11. Zebec, V.: Mikrobiološka kvaliteta mlijeka u proizvodnji sira od sirovog mlijeka. (Microbiological quality of milk in the production of cheese from raw milk.) [Doctoral dissertation.] Zagreb : University of Zagreb, 2016. <https://urn.nsk.hr/urn:nbn:hr:204:297272> In Croatian.
12. Tandir, S.: Zdravstvena ispravnost travnickog sira i standardi kvaliteta higijenske ispravnosti. (Health accuracy of Travnik cheese and quality standards of sanitary accuracy.) Medicinski Arhiv, *59*, 2005, pp. 326–327. ISSN: 0350-199X. In Bosnian.
13. Alagić, D. – Smajlović, M. – Članjak, E. – Čaklovica, K.: *Staphylococcus aureus* – patogen prenosiv hranom. (*Staphylococcus aureus* – foodborne pathogen). Sarajevo : Veterinarski fakultet Sarajevo, 2015. ISBN: 978-9958-599-58-3. In Bosnian.
14. ISO 7218:2024. Microbiology of the food chain – General requirements and guidance for microbiological examinations. Geneva : International Organization for Standardization, 2024.
15. ISO 6887-5:2020. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products. Geneva : International Organization for Standardization, 2020.
16. ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. Geneva : International Organization for Standardization, 2017.
17. ISO 11290-1:2017. Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method. Geneva : International Organization for Standardization, 2017.
18. ISO 6888-1:2021/Amd 1:2023. Microbiology of the food chain – Horizontal method for the enumeration of coagulase-positive staphylococci (S*taphylococcus aureus* and other species) – Part 1: Method using Baird-Parker agar medium. Geneva : International Organization for Standardization, 2021.
19. ISO 21528-2:2017. Microbiology of the food chain – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count technique. Geneva : International Organization for Standardization, 2017.
20. ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 °C by the pour plate technique. Geneva : International Organization for Standardization, 2013.
21. ISO 16649-2:2001. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. Geneva : International Organization for Standardization, 2001.
22. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95. Geneva : International Organization for Standardization, 2008.
23. Carafa, I – Clementi, F – Tuohy, K – Franciosi, E.: Microbial evolution of traditional mountain cheese and characterization of early fermentation cocci for selection of autochthonous dairy starter strains. Food Microbiology, *53*, Part B, 2016, pp. 94–103, DOI: 10.1016/j.fm.2015.09.001.
24. Künz, J. J. – Roch, F.-F. – Dzieciol, M. – Sofka, D. – Hilbert, F. – Selberherr, E.: Microbial dynamics in the ripening process of Vorarlberger Alpkäse: farm-specific variations at two Austrian alpine farms. Frontiers in Microbiology, *16*, 2025, article 1617995. DOI: 10.3389/fmicb.2025.1617995.
25. Kochetkova, T. V. – Grabarnik, I. P. – Klyukina, A. A. – Zayulina, K. S. – Gavirova, L. A. – Shcherbakova, P. A. – Kachmazov, G. S. – Shestakov, A. I. – Kublanov, I. V. – Elcheninov, A. G.: The bacterial microbiota of artisanal cheeses from the Northern Caucasus. Fermentation, *9*, 2023, article 719. DOI: 10.3390/fermentation9080719.
26. Smit, G.: Dairy processing: improving quality. Cambridge : Woodhead Publishing, 2003. ISBN: 978-1-85573-676-4.
27. Samaržija, D. – Zamberlin, Š. – Pogačić, T.: Psychrotrophic bacteria and their negative impact on milk and dairy product quality. Mljekarstvo, *62*, 2012, pp. 77–95. ISSN: 0026-704X. <https://hrcak.srce.hr/file/124020>
28. Panelli, S. – Brambati, E. – Bonacina, C. – Feligini, M.: Diversity of fungal flora in raw milk from the Italian Alps in relation to pasture altitude. SpringerPlus, *2*, 2013, article 405. DOI: 10.1186/2193-1801-2-405.
29. Oliveira, W. C. – de Freitas, A. S. – Ströher, J. A. – Richards N. S. P. D. S. – Oliveira, M. B. P. P. – Erhardt, M. M.: Exploring the impact of altitude on bacterial communities in informally produced artisanal colonial cheeses: insights from 16S rRNA gene sequencing. Microorganisms, *13*, 2025, article 1116. DOI: 10.3390/microorganisms13051116.
30. Službeni glasnik BiH, broj 11/13. Pravilnik o mikrobiološkim kriterijima za hranu. (Official Gazette of Bosnia and Herzegovina, number 11/13. Rulebook on microbiological criteria for food). In: Službeni List BiH [online]. Sarajevo : Službeni List Bosne i Herzegovine, 2013 [accessed 21 January 2026]. <http://www.sluzbenilist.ba/page/akt/5rC0A9eZvTA=> In Bosnian.

31. De Respinis, S. – Caminada, A. – Pianta, E. – Buetti-Dinh, A. – Scettrini, P. R. – Petrini, L. – Tonolla, M. – Petriniet, O.: Fungal communities on alpine cheese rinds in Southern Switzerland. Botanical Studies, *64*, 2023, article 6. DOI: 10.1186/s40529-023-00371-2.
32. Bintsis, T.: Yeasts in different types of cheese. AIMS microbiology, *7*, 2021, pp. 447–470. DOI: 10.3934/microbiol.2021027.
33. Kumar, S. – Suyal, D. C. – Yadav, A. – Shouche, Y. – Goel, R.: Microbial diversity and soil physiochemical characteristic of higher altitude. PLOS ONE, *14*, 2019, article e0213844. DOI: 10.1371/journal.pone.0213844.
34. *E. coli*. In: World Health Organization [online]. Geneva : World Health Organization, 7 February 2018 [accessed 21 January 2026]. <https://who.int/newsroom/fact-sheets/detail/e-coli>
35. Praça, J. – Furtado, R. – Coelho, A. – Correia, C. B. – Borges, V. – Gomes, J. P. – Pista, A. – Batista, R.: *Listeria monocytogenes, Escherichia coli* and coagulase positive staphylococci in cured raw milk cheese from Alentejo region, Portugal. Microorganisms, *11*, 2023, article 322. DOI: 10.3390/microorganisms11020322.
36. Alrhmoun, M. – Zanon, T. – Katzenberger, K. – Holighaus, L. – Gauly, M.: Exploring the heights: Impact of altitude on dairy milk composition. JDS Communications, *5*, 2024, pp. 139–143, DOI: 10.3168/jdsc.2023-0448.

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