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Effects of Hygiene on Milk Quality and Milking Practices of Small Andean Herds during the Rainy Season

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ABSTRACT

Dairy production in the Peruvian Andes is mainly based on small herds. However, there is little information on how hygiene affects milk quality during the rainy season. The study's objective was to evaluate the hygienic factors of milk and milking practices during the rainy season in small herds located at two high Andean altitudinal levels. The characteristics of the factors related to the milking process were recorded using an observation guide, and 108 raw milk samples were collected from 18 herds with Brown Swiss cows at two altitude levels. Samples were obtained from the milk collection containers and milk. The results showed that 56.5% of the samples analyzed were unhygienic, with no significant differences in bacterial counts between altitudinal levels. However, a strong correlation was identified between precipitation and the presence of coliforms (0.726) and mesophilic aerobes (0.861). Factors such as milking location, hand washing, and use of cleaning agents were associated with microbial contamination, acquiring odds ratios (OR) of 4.04, 5.26, and 4.71, respectively, during the months of heavy rain. The study concludes that the hygienic quality of milk in small high Andean herds significantly deteriorates during the rainy season, with counts of total coliform bacteria and mesophilic aerobes exceeding recommended levels, particularly during peak rainfalls. This finding highlights a direct relationship between the intensity of rainfall and the quality of milk, underscoring the need for improved milking practices in the rainy season to ensure the safety of the products.

Keywords: Andean ecosystem, Milk quality, Rainy season, Small farmer

INTRODUCTION

The hygienic quality of milk is a fundamental aspect of production, as milk is an essential component of the population's daily diet. Ensuring that milk is safe is crucial for meeting basic nutritional needs and ensuring the consumers' overall well-being. Milk provides essential nutrients such as proteins, calcium, and vitamins, which are vital for development and health at all stages of life. However, its susceptibility to microbial contamination can compromise its quality and safety (Boor et al., 2017). It is essential to understand that milk, as a biological product, can be a vehicle for pathogens if not handled under strict hygienic standards (Owusu-Kwarteng et al., 2020).

Internationally, various studies have identified the main factors influencing the hygienic quality of raw milk. Research in Latin America, Africa, and Asia has shown that milking hygiene, udder cleanliness, and milker hygiene are critical factors in reducing contamination by total coliform bacteria and mesophilic aerobic bacteria (Múnera-Bedoya et al., 2017; Bereda et al., 2018; Kazeminia et al., 2023). Additionally, the presence of zoonotic pathogens such as *Escherichia coli* and *Salmonella* in raw milk has been correlated with inadequate milking practices, highlighting the importance of improving sanitary conditions in dairy farms (Geletu et al., 2022). However, in countries like Canada, small farms employ good milking practices, conducting monthly sampling to detect pathogenic microorganisms and indicator bacteria to ensure milk safety and quality (Berge and Baars, 2020). In contrast, in developing countries, non-compliance with quality standards often forces dairy producers into the unofficial market (Candiotto et al., 2020).

However, some studies on milk quality in intensive and semi-extensive production systems have been conducted, such as those by Fuentes et al. (2014) and Chagray et al. (2023). Notwithstanding, small dairy producers in high Andean areas have received less scientific attention. This lack of focus is concerning, as adverse weather conditions—such as wind, rain, and fog—affect the hygienic conditions of animals, milkers, and the milking process, thereby increasing the

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chances of contamination. Prolonged rainfall may promote the proliferation of microorganisms in corrals and during milking, increasing the risk of cross-contamination between the environment, animals, and the final product (Candiotto et al., 2020).

The scientific gap lies in the lack of research that directly links milking practices and the hygienic quality of milk in small dairy herds, especially in the Peruvian highlands. This study assesses the hygienic quality of milk by measuring the total coliform bacteria (TCB) and mesophilic aerobic bacteria (MBB) through the Compact Dry methods (Nissui Pharmaceutical, 2019), alongside an examination of the factors affecting manual milking during the rainy season in small dairy herds located in the high Andean region of central-southern Peru. Therefore, the study aimed to evaluate the hygienic quality of milk and milking practices during the rainy season in small herds located at two high Andean altitudinal levels.

MATERIALS AND METHODS

Ethical approval

The study received approval from the Ethics Committee of the National University of San Cristóbal de Huamanga (Peru) with Letter No. 01-CE-VRI-UNSCH-2024. The research was carried out taking into consideration the Code of Ethics of the Peruvian Veterinary Medical College (s.f.), on the promotion and protection of animal health and well-being, public health, and environmental conservation (Art. 10) and on conducting research with living animals, trying to avoid physical suffering and stress (Art. 85).

Study area

The study was conducted in the Ayacucho region of Peru, where two ecological levels were identified based on altitude (Aybar and Lavado, 2017), the upper montane level (UMZ), between 3800 and 3920 meters above sea level (masl) in the Socos district, and the lower montane level (LMZ), ranging from 3350 to 3500 masl in the Chiara district. In this area, 58 small dairy herds of Brown Swiss cows were identified, with 27 herds in the upper part and 31 in the lower part. From these, 9 herds were selected from each level. The inclusion criteria were accessibility and having at least 4 cows in production (Figure 1). The fieldwork was conducted between October 2023 and March 2024, corresponding with the start and end of the rainy season (Table 1).

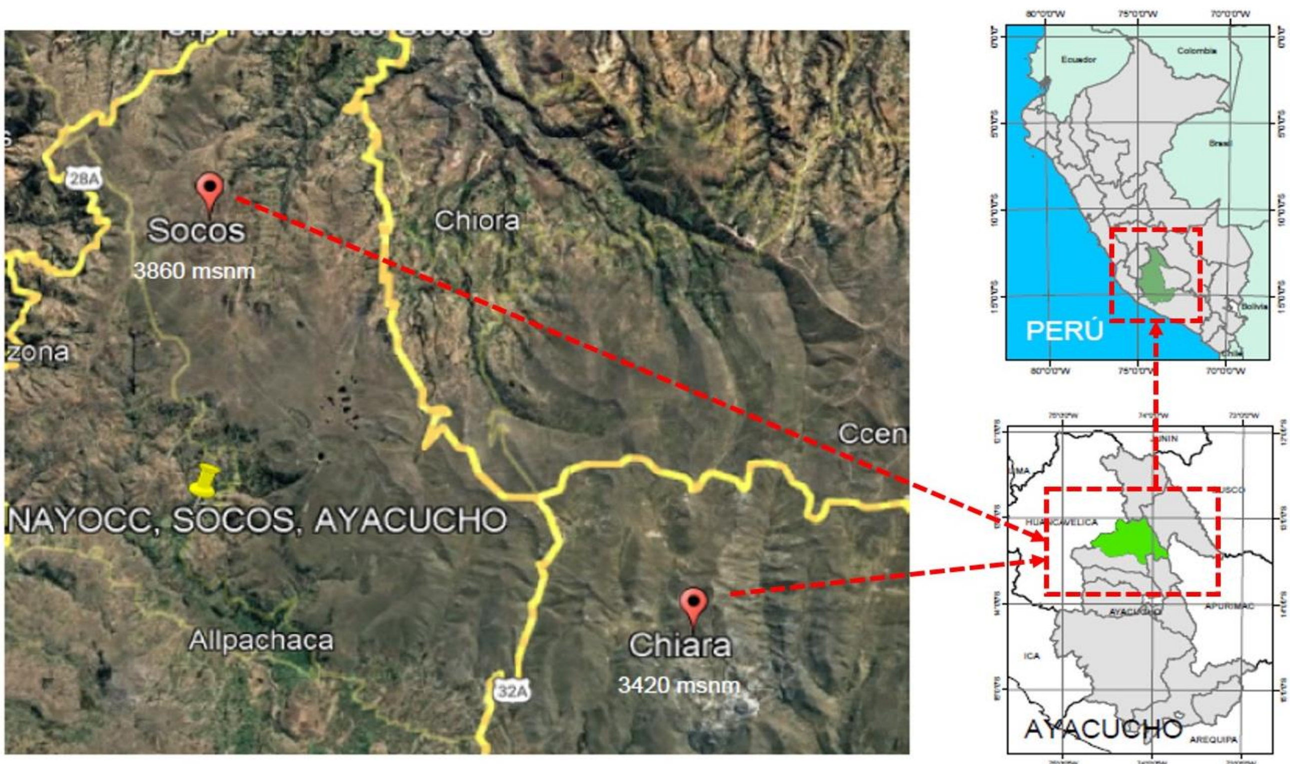

Figure 1. The location of the study in Peru, Ayacucho region, Socos, and Chiara districts

Table 1. Rainfall during the study period in Huamanga, Ayacucho, Peru

Weather conditions	2023			2024		
(average)	October	November	December	January	February	March
Precipitation (mm)	70	89	126	140	130	94
Temperature (°C)	15	15	15	15	14	14
Humidity (%)	78	81	86	88	89	86

Sources: Weather-Atlas (2023-2024) and Senamhi (2023).

Observation of milking factors

The characteristics of the manual milking process in the absence of infrastructure were recorded using an observation guide. The guide converged on the sex of the milkers, milking location, hand washing, use of a mask, udder cleaning, and cleaning of utensils. The use of cleaning agents such as soap for hand washing and udder cleaning, as well as detergents for cleaning utensils, was also observed.

Sampling

Milk samples were collected from the collection containers, and the product of the day's milking was stored in the herd between 8:00 and 9:00 a.m. Approximately 500 ml of milk was obtained for each sample using sterile polypropylene containers (Pantoja et al., 2011). Subsequently, the samples were refrigerated at 4°C and transported to the laboratory within 6 hours of collection (Kiambi et al., 2022).

Laboratory analysis

The analyses were performed at the Microbiology Laboratory of the National University of San Cristóbal de Huamanga (Ayacucho, Peru). The samples were analyzed to determine the presence of total coliform bacteria (TCB) and aerobic mesophilic bacteria (AMB), using the rapid colony count method with Compact DryTM plates (Nissui Fharmaceutical, 2019). The procedure included the preparation of a 0.1% peptone broth, the subsequent preparation of serial dilutions (10^{-3} dilutions for total coliforms and 10^{-4} for mesophilic aerobes), followed by the inoculation of the milk samples on Compact Dry EC plates for total coliforms and on TC plates for mesophilic aerobes (2 replicates per sample), and finally the incubation of Compact Dry EC plates at 37°C for 24 hours and TC plates at 37°C for 48 hours. The results were expressed in CFU/ml according to the manufacturer's recommendations (Nissui Pharmaceutical, 2019) and evaluated according to NTP.202.001 (Peruvian Technical Standard, 2016).

Statistical analysis

Data were tabulated and statistically analyzed to calculate measures of central tendency and dispersion for total coliform bacteria (TCB) and aerobic mesophilic bacteria (AMB) counts. Spearman correlation analysis was used to assess relationships while Analysis of Variance (ANOVA) and Tukey's significance test ($p < 0.05$) were used to identify statistical differences. In addition, logistic regression was applied to determine odds ratios. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24.

RESULTS AND DISCUSSION

Total coliform bacteria

Total coliform bacteria count (CFU/ml) varied significantly, reaching their highest value in January, both in the lower montane and upper montane altitudinal levels ($p < 0.05$). In both altitudinal levels, the variation in the bacterial counts followed a similar trajectory; at the beginning of the study in October, the total coliform counts were low, with a progressive increase until January, followed by a gradual decrease until March. Statistical comparison between the two altitudinal levels showed no significant differences in coliform counts, indicating a consistent pattern in both zones ($p > 0.05$). A high percentage of raw milk samples in both herds, located in the lower montane and the upper montane altitudinal levels, showed contamination with total coliform bacteria. According to the Peruvian technical standard, on average, only 43.5 % of the samples met the safety standards showing no contamination (PTS, 2016).

A correlation analysis was conducted to assess the association between variations in total coliform bacteria counts (Table 2) and the intensity of monthly rainfall (Table 1), which indicated a significant relationship ($r = 0.726$, $p < 0.05$). The analysis showed a strong and statistically significant positive correlation between rainfall and total coliform bacteria counts ($p < 0.05$). The findings indicate that increased humidity levels resulting from rainfall may promote the growth of

coliform bacteria within livestock populations, highlighting the critical need for effective sanitary management during the rainy season. Additionally, the data reveal a correlation between the total coliform bacteria counts and the intensity of rainfall. The results are consistent with earlier studies that demonstrate seasonal fluctuations in coliform levels in hand-milked herds, showing elevated counts during colder and wetter seasons (De Garnica et al., 2013). Other studies have noted positive correlations between temperature, humidity, and somatic cell counts (Bertocchi et al., 2014) as well as seasonal patterns, in which total coliform bacteria counts are typically lowest in winter (Bokharaeian et al., 2023).
Total coliform bacteria, once released from cow feces, tend to disseminate within the environment, thereby establishing a transmission cycle (Perdomo and Calle, 2024). These bacteria not only compromise the hygienic quality of milk but also pose a significant risk to public health (Rahman et al., 2020). The lack of variation in total coliform bacteria counts across different altitudinal levels indicates uniform conditions regarding bacterial load. Furthermore, the identified positive correlation between rainfall and coliform counts is consistent with the observations made by Bokharaeian et al. (2023), who also reported a relationship between humidity levels and microbial load that can adversely affect milk quality.

Table 2. The total coliform bacteria (CFU/ml) count, according to altitudinal level and months of the year

Months	Lower montane altitudinal level		Upper montane altitudinal level		Overall
	Mean		Mean		
October	$1.83×10^3$	b	$1.11×10^3$	b	$1.47×10^3$
November	$1.67×10^3$	ab	$3.11×10^3$	b	$2.39×10^3$
December	$3.28×10^3$	ab	$9.17×10^3$	ab	$6.22×10^3$
January	$3.07×10^4$	a	$4.24×10^4$	a	$3.66×10^4$
February	$8.51×10^3$	ab	$1.16×10^4$	ab	$1.01×10^4$
March	$5.06×10^3$	ab	$2.22×10^3$	b	$3.64×10^3$
p-value[f]	0.008		0.001		
p-value[g]	0.198				
The percentage of unhygienic samples that do not follow PTS	55.5 %		57.5 %		56.5 %
Relationship between TCB and rainfall: r = 0.726; confidence interval= 0.616 – 0.999; Bilateral sig. = 0.042					

[a,b] Different superscript letters in the same column differ significantly at 95%. PTS: Peruvian technical standard (PTS: 202.001). TCB: Total coliform bacteria, Sig: Significant; [f] Comparison of the total coliform bacteria in each altitudinal floor, [g] Comparison of the total coliform bacteria between the altitudinal floors

Aerobic mesophilic bacteria

The measurements of mesophilic aerobic bacteria (CFU/ml) displayed a trend consistent with that of total coliform bacteria, showing considerable monthly variations and attaining a maximum in January at both lower and upper montane altitudinal levels ($p < 0.05$). At both altitudinal levels, the variation in counts followed a comparable trajectory, in which mesophilic aerobic bacteria counts were initially low at the beginning of the study in October, progressively increased until January, and then gradually declined until March. A statistical comparison between the altitudinal levels indicated no significant differences, suggesting that the bacterial counts were similar across both levels ($p > 0.05$). A large proportion of raw milk samples in both regions showed contamination by mesophilic aerobic bacteria, with 56.3% meeting safety standards as indicated by Peruvian technical standards (PTS, 2016).

A correlation analysis was performed to determine if the variation in mesophilic aerobic bacteria counts (Table 3) is related to the intensity of monthly rainfall (Table 1), which indicated a strong and significant relationship ($r = 0.861$, $p < 0.05$). The results indicate a strong and statistically significant positive correlation between rainfall and mesophilic aerobic bacteria counts. The findings indicate that increased humidity levels resulting from rainfall may promote the growth of mesophilic aerobic bacteria in livestock populations, which is significant for health management practices during the rainy season. The observed aerobic mesophilic bacteria count (CFU/ml) aligns with rainfall intensity. Other studies report that temperature and humidity are positively associated with somatic cell count (Bertocchi et al., 2014), that mesophilic colonies were lower in winter and higher in summer (Petróczki et al., 2020), and that changes in seasons, months, and temperature and humidity indices affect both milk production and quality (Bokharaeian et al., 2023).

The techniques used during milking are linked to aerobic mesophilic bacteria counts (Ngolombe et al., 2024), with lower counts reported in colder seasons (Kazeminia et al., 2023); however, poor farm hygiene remains a significant factor compromising milk quality and safety (Nyokabi et al., 2021). The similarity in CFU/ml counts across altitudinal levels suggests the existence of comparable conditions for bacterial load across both regions. In addition, the positive correlation between rainfall and aerobic mesophilic bacteria counts corroborates findings by Bokharaeian et al. (2023), which also pointed to a close relationship between humidity indices and microbial load. Improved milking practices can play a vital role in enhancing milk's hygienic quality (Lemma et al., 2018).

Table 3. The aerobic mesophilic bacteria (CFU/ml) count, according to altitudinal level and months of the year

Months	Lower montane altitudinal level	Upper montane altitudinal level	Overall
	Mean	Mean	
October	4.96×10^5 c	5.98×10^5 c	5.47×10^5
November	6.04×10^5 bc	7.73×10^5 bc	6.89×10^5
December	1.65×10^6 ab	1.20×10^6 ab	1.42×10^6
January	3.05×10^6 a	1.92×10^6 a	2.48×10^6
February	1.09×10^6 b	8.71×10^5 bc	1.22×10^6
March	1.38×10^6 bc	1.07×10^6 bc	9.79×10^5
p-value[f]	0.001	0.001	
p-value[g]	0.982		
The percentage of unhygienic samples that do not follow PTS	43.1 %	44.3 %	43.7 %
Relationship between MAB and rainfall: r = 0.861; confidence interval= 0.731 – 0.999; Bilateral sig. = 0.028			

[a,b,c] Different superscript letters in the same column differ significantly at 95%. PTS: Peruvian technical standard (PTS: 202.001). MAB: mesophilic aerobic bacteria, Sig: Significant; [f] comparison of the mesophilic aerobic bacteria in each altitudinal floor,[g] comparison of the mesophilic aerobic bacteria between the altitudinal floors

Table 4. Different factors associated with milking, in months with low and high precipitation, according to altitudinal levels

Factor	Variable	In months of less rainfall				In months of greater rainfall				p-value
		LMF		UMF		LMF		UMF		
		n	(%)	n	(%)	n	(%)	n	(%)	
Gender	Woman	24	88.9	23	85.2	24	88.9	22	81.5	0.698
	Man	3	11.1	4	14.8	3	11.1	5	18.5	
Milking place	Clean	18	66.7	10	37.0	19	70.4	10	37.0	0.941
	No clean	9	33.3	17	63.0	8	29.6	17	63.0	
Handwashing	Yes	21	77.8	15	55.6	25	92.6	19	70.4	0.445
	No	6	22.2	12	44.4	2	7.4	8	29.6	
Hand washing cleaning agent	Water	24	88.9	21	77.8	24	88.9	17	63.0	0.652
	Water and soap	3	11.1	6	22.2	3	11.1	10	37.0	
Use of mask	Yes	16	59.3	13	48.1	17	63.0	10	40.7	0.895
	No	11	40.7	14	51.9	10	37.0	16	59.3	
Cleaning udders	Yes	17	63.0	15	55.6	18	66.7	12	44.4	0.782
	No	10	37.0	12	44.4	9	33.3	15	55.6	
Udder cleaning agent	Water	21	77.8	20	74.1	23	85.2	18	66.7	0.938
	Water and soap	6	22.2	7	25.9	4	14.8	9	33.3	
Cleaning utensils	Yes	18	66.7	17	63.0	19	70.4	13	48.1	0.843
	No	9	33.3	10	37.0	8	29.6	14	51.9	
Cleaning agent for utensils	Water	24	88.9	23	85.2	21	77.8	14	51.9	0.420
	Water and detergent	3	11.1	4	14.8	6	22.2	13	48.1	

* LMF: Lower montane altitudinal floor; UMF: Upper montane altitudinal floor. N: Number

Factors associated with milking

All the factors associated with milking observed in herds on both the lower and upper montane altitudinal floors as well as in the months with the lowest and highest rainfall (Table 4) showed similar characteristics ($p > 0.05$). Logistic regression analysis revealed specific risk factors that may serve as indicators for milk contamination, as evidenced by their elevated odds ratio (OR) values. In the months characterized by minimal rainfall (October-November), the factors of milking location, hand washing practices, and the application of cleaning agents for hand washing exhibited ORs of

3.40, 2.80, and 2.29, respectively; however, these values did not achieve statistical significance ($p > 0.05$). Conversely, during the months with the highest levels of rainfall (December-January), these factors demonstrated a notable increase in their OR values, reaching 4.04, 5.26, and 4.71 ($p < 0.05$), indicating an elevated risk. Furthermore, the utilization of cleaning agents for milking utensils emerged as an additional concern during these months, with an OR of 3.25 ($p < 0.05$), highlighting an increased risk associated with higher rainfall conditions (Table 5).

The increase in ORs for several milking-associated factors during high-rainfall months suggests a direct impact of rainfall on suitable conditions for milking during the rainy season. This finding is consistent with the results of studies by Paraffin et al. (2018), Alaru et al. (2022), and Deddefo et al. (2023), which have documented how high rainfall increases microbiological contamination due to the difficulty of maintaining hygienic practices in humid and unhygienic environments. Likewise, studies by Nyokabi et al. (2021) and Xulu and Naidoo (2023) highlight the need for adequate cleaning and disinfection of utensils as a fundamental practice to ensure the microbiological safety of milk.

Table 5. Logistic regression of different factors associated with milking in cows

Factor	In months of less rainfall			In months of greater rainfall		
	OR	CI	p-value	OR	CI	p-value
Gender	1.04	0.19 – 5.71	0.687	1.45	0.29 – 7.24	0.478
Milking place	3.40	1.11 – 10.40	0.280	4.04	1.30 – 12.59	0.014
Handwashing	2.80	0.86 – 9.14	0.074	5.26	1.00 – 27.69	0.038
Hand washing cleaning agent	2.29	0.51 – 10.29	0.234	4.71	1.12 – 19.70	0.027
Use of mask	1.57	0.53 – 4.60	0.290	2.47	0.83 – 7.39	0.086
Cleaning udders	1.36	0.46 – 4.04	0.391	2.50	0.83 – 7.53	0.085
Udder cleaning agent	1.23	0.35 – 4.28	0.500	2.88	0.76 – 10.87	0.101
Cleaning utensils	1.18	0.39 – 3.60	0.500	2.56	0.84 – 7.83	0.083
Cleaning agent for utensils	1.39	0.28 – 6.91	0.500	3.25	1.00 – 10.58	0.043

OR: Odds ratio; CI: confidence interval.

CONCLUSION

The rainy season significantly affects the hygienic quality of milk produced by small herds in the high Andes. During this period, total coliform and mesophilic aerobic counts surpass the recommended limits, especially in December and January, which coincide with the highest levels of rainfall. This observation highlights the direct correlation between the intensity of rainfall and the quality of milk. Three key factors related to the milking process were identified as impacting the hygienic quality of the milk including the location of milking, the practices of handwashing, and the application of cleaning agents for both handwashing and utensils. Addressing these elements is essential for maintaining adequate hygienic standards, indicating the priority attention they require. The findings highlight the urgency of implementing programs to improve hygiene practices during the rainy season. Such programs include focusing on training producers, providing adequate cleaning supplies, and improving milking infrastructure.

DECLARATIONS

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Availability of data and materials

Data of the present study is available upon reasonable request.

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Authors' contributions

Gloria Adrianzen, Julio Ruiz, and Nelly Umppiri planned the experiment and performed the fieldwork. Francisco Espinoza interpreted and analyzed the data to draft the manuscripts. All authors reviewed and approved the final edition of the manuscript.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

Before submitting their study to the journal, all authors thoroughly checked and confirmed ethical concerns regarding the originality of the data collected and analyzed.

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