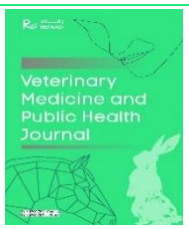
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Assessment of Microbial Quality of Raw Milk in Nangarhar Province, Afghanistan

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Abstract:

Objectives: This paper aims to evaluate the microbiological quality of raw milk in Nangarhar Province, Afghanistan, and to identify factors influencing its safety. Despite widespread consumption of raw milk in the districts, limited data exist at the district level regarding bacterial contamination and potential public health risks.

Methods: A descriptive cross-sectional study was conducted, in which 80 raw milk samples were collected from cow and buffalo sources across four districts during different seasons. Standard microbiological methods were employed to determine total bacterial load and to detect coliform bacteria and Escherichia coli as indicators of hygiene and potential health hazards. The study assessed the influence of animal source, seasonal variation, and local handling practices on milk contamination.

Results: Considerable variations in microbial contamination were observed among districts, seasons, and milk sources. Higher bacterial counts were recorded during warmer periods compared to winter, and cow milk showed greater contamination than buffalo milk. Districts with poor sanitation and inadequate handling practices exhibited higher microbial loads. The presence of hygiene-related bacteria in a significant proportion of samples indicates suboptimal milking, storage, and transportation conditions.

Conclusion: Consumption of raw milk in the study area may pose public health risks. The study underscores the need for improved hygienic practices, farmer awareness programs, and routine monitoring of milk quality. These findings provide baseline data to support food safety planning and future research on microbiological quality of raw milk in Nangarhar Province.

Keywords: *Raw milk; microbiological quality; bacterial contamination; coliform bacteria; Escherichia coli; milk hygiene; Afghanistan.*

1 Introduction

Milk is a vital food commodity consumed worldwide, providing essential nutrients such as high-quality proteins, fats, carbohydrates, vitamins, and minerals that support growth, development, and overall health across all age groups (Yuan et al., 2022).It is naturally secreted by the mammary glands of mammals and is primarily obtained from cows, buffaloes, goats, and sheep, with cow and buffalo milk representing the largest share of global production. Beyond nutrition, milk and dairy products hold significant cultural and economic value in many regions (Ahmadi, 2025; Khan et al., 2022).

Milk consists mainly of water and solids, including fat, proteins (casein and whey), lactose, vitamins, and minerals such as calcium and phosphorus. These components are critical for bone development, metabolic functions, and overall nutrition (Ahmed, 2022; Swai & Schoonman, 2011). However, milk composition is not constant and varies with animal breed, feeding practices, physiological condition, environmental factors, and seasonal changes. Seasonal variation influences milk yield and composition, affecting both quality and suitability for processing (Martin et al., 2023; Deddefo et al., 2023).

Despite its nutritional benefits, raw milk is highly perishable and prone to microbial contamination during milking, handling, storage, and transportation. Poor hygiene, inadequate cooling facilities, and substandard infrastructure increase bacterial growth risks. Coliform bacteria and Escherichia coli are commonly used as indicators of sanitation and potential public health hazards. Consumption of contaminated raw milk has been associated with foodborne illnesses, highlighting milk safety as a critical public health concern (McLauchlin et al., 2020; Swai & Schoonman, 2011).

Previous studies indicate that microbial contamination varies across districts and seasons, with warmer periods favoring bacterial growth. However, district-level assessments in Nangarhar Province, Afghanistan, remain limited, and comparative data on cow versus buffalo milk under local production conditions are scarce (Knight-Jones et al., 2016; Mshana et al., 2025).

This study aims to evaluate the microbial quality of raw milk collected from different districts of Nangarhar Province by assessing total bacterial load and hygiene-related indicator organisms in cow and buffalo milk, while examining the influence of seasonal variation and local handling practices. The findings provide baseline data to support food safety policies, hygiene interventions, and public health protection in the region.

2 Materials and Methods

2.1 Research Design and Study Area

A descriptive cross-sectional study was conducted to evaluate the microbial quality of raw milk collected from different districts of Nangarhar Province, Afghanistan. The study aimed to assess bacterial contamination in raw cow and buffalo milk and to examine the effect of seasonal variation on microbial load.

2.2 Sample Collection

A total of 80 raw milk samples were collected from four districts of Nangarhar Province: Bihsud, Goashta, Bati Kot, and Kama. From each district, ten cow milk samples and ten buffalo milk samples were collected per season. Sampling was conducted during two seasons: winter and spring, resulting in 40 samples per season (20 cow milk and 20 buffalo milk). Milk samples were collected in sterile, airtight plastic bottles, properly labeled, and transported to the laboratory under refrigerated conditions at 4 °C.

Table 1: Sampling Plan

Bihsud	Sc	5
	Sb	5
Goshta	Sc	5
	Sb	5
Bati Kot	Sc	5
	Sb	5
Kama	Sc	5
	Sb	5

Sc: Sample of Cows, Sb: Sample of Buffaloes

2.3 Sample Handling and Preparation

Upon arrival at the laboratory, samples were stored at 4 °C and analyzed within 24 hours. Prior to analysis, samples were thoroughly mixed to ensure homogeneity. Serial dilutions were prepared using sterile normal saline solution (0.85–0.89% NaCl) according to standard microbiological procedures.

2.4 Microbiological Analysis

Microbial quality assessment included Total Plate Count (TPC), Total Coliform Count (TCC), and Escherichia coli enumeration using standard culture-based methods:

Total Plate Count (TPC): Determined using Nutrient Agar to estimate the total viable bacterial load. Plates were incubated at 37 °C for 24 hours, and colonies were counted from plates containing 30–300 colonies.

Total Coliform Count (TCC): Determined using Eosin Methylene Blue (EMB) agar. Plates were incubated at 35–37 °C for 24 hours, and characteristic coliform colonies were enumerated.

Escherichia coli: Detected and quantified using MacConkey agar, a selective and differential medium for lactose-fermenting gram-negative bacteria. Plates were incubated at 37 °C for 48 hours, and typical pink to red colonies were counted.

Microbial counts were expressed as logarithmic colony-forming units per milliliter (log CFU/mL).

2.5 Statistical Analysis

Data from microbiological analyses were statistically analyzed using a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was applied to determine the effects of district, season, and milk source on microbial counts. Mean values were compared using Tukey's test at a significance level of $p < 0.05$. Statistical analyses were performed using Statistic version 8.1, following standard procedures described by Montgomery (2017).

3 Results

The microbial quality of raw milk samples collected from different districts of Nangarhar Province showed noticeable variations between winter and spring seasons, as well as between cow and buffalo milk.

3.1 Total Plate Count (TPC)

As shown in Table 2 and Figure 1, the Total Plate Count (TPC) of raw milk samples varied considerably across districts and seasons. Generally, higher TPC values were observed during the spring season compared to winter. The highest TPC value was recorded in Kama district for cow milk during spring (65.64 $\times 10^5$ CFU/mL), while the lowest value was observed in Goashta district for cow milk during winter (5.65 $\times 10^5$ CFU/mL). This seasonal increase in microbial load may be associated with higher environmental temperatures and increased microbial growth during spring.

3.2 Total Coliform Count (TCC)

The Total Coliform Count (TCC) also showed seasonal variation (Figure 2). Higher coliform counts were generally recorded in spring samples, indicating possible contamination during milk handling and transportation. The highest TCC value was observed in Kama district cow milk during spring (24.92 $\times 10^5$ CFU/mL), whereas the lowest value was found in buffalo milk from Bihsud district during winter (1.74 $\times 10^5$ CFU/mL) (Parvin, Begum, & Buragohain, 2017), indicating unsatisfactory hygienic conditions during warmer months.

3.3 Escherichia coli Count

The presence of E. coli in raw milk samples indicates possible fecal contamination and poor hygienic practices during milking and handling. As illustrated in Table 2 and Figure 3, E. coli counts were generally higher in spring samples compared to winter. The highest E. coli count was observed in Kama district cow milk during spring (19.66 $\times 10^5$ CFU/mL), while the lowest value was found in buffalo milk from Bati Kot during winter (2.03 $\times 10^5$ CFU/mL) recommended by Quigley et al. (2013), suggesting a higher risk of fecal contamination.

Overall, the results indicate that seasonal factors and hygiene conditions during milk production and handling may significantly influence the microbial quality of raw milk in the study area.

3.4 Seasonal and Regional Trends

A key finding was the universal trend of elevated microbial loads in spring compared to winter across all districts. While values occasionally differed between cow and buffalo milk within the same district and season, the seasonal effect remained dominant. For example, in Bati Kot, cow milk TPC did not change significantly between seasons, whereas buffalo milk and all other districts exhibited significant seasonal increases.

Table 2: Mean Microbial Counts (×10⁵ CFU/mL± SD) in Raw Milk Samples across District and Seasons

District	Animal	Season	TPC(×10⁵ CFU/mL)	TCC(×10⁵ CFU/mL)	E. Coli(×10⁵ CFU/mL)
Bihsud	Cow	Winter	15.2 ±1.51	3.19 ±0.93	3.40 ±1.18
		Spring	41.52 ±4.41	7.15 ±1.17	7.55 ±0.98
Bihsud	Buffalo	Winter	13.55±0.72	1.74±1.74	1.75±1.75
		Spring	39.25±5.24	7.68±0.50	8.08±0.49
Goshta	Cow	Winter	5.65 ±0.15	2.34 ±0.20	2.30 ±0.17
		Spring	26.88 ±0.59	6.78 ±0.48	6.82 ±0.17
Goshta	Buffalo	Winter	5.80±1.83	2.34±0.20	2.30±1.17
		Spring	33.6±5.16	6.78±0.48	5.86±0.44
Bati Kot	Cow	Winter	14.70 ±0.39	14.71 ±0.39	3.23 ±2.04
		Spring	14.71 ±1.27	7.02 ±0.70	7.67 ±0.74
Bati Kot	Buffalo	Winter	14.22±0.42	4.77±0.17	2.03±0.13
		Spring	28.65±1.59	16.78±1.21	11.35±0.55
Kama	Cow	Winter	5.36 ±0.12	2.28 ±0.46	3.51 ±0.70
		Spring	65.64 ±6.00	24.92 ±2.98	19.66 ±1.16
Kama	Buffalo	Winter	5.13±0.16	2.18±0.08	2.37±0.14
		Spring	26.16±2.06	9.12±1.07	7.21±0.60

Note: *TPC = Total Plate Count; TCC = Total Coliform Count.*

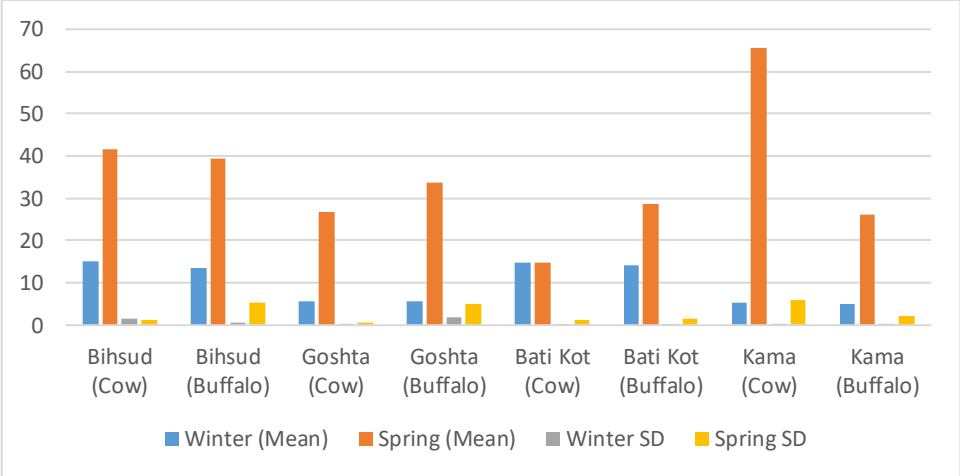


Figure 1: Seasonal Variation in Total Plate Count (TPC) in raw milk samples across districts and animal types. Unit: CFU/mL ×10⁵±SD

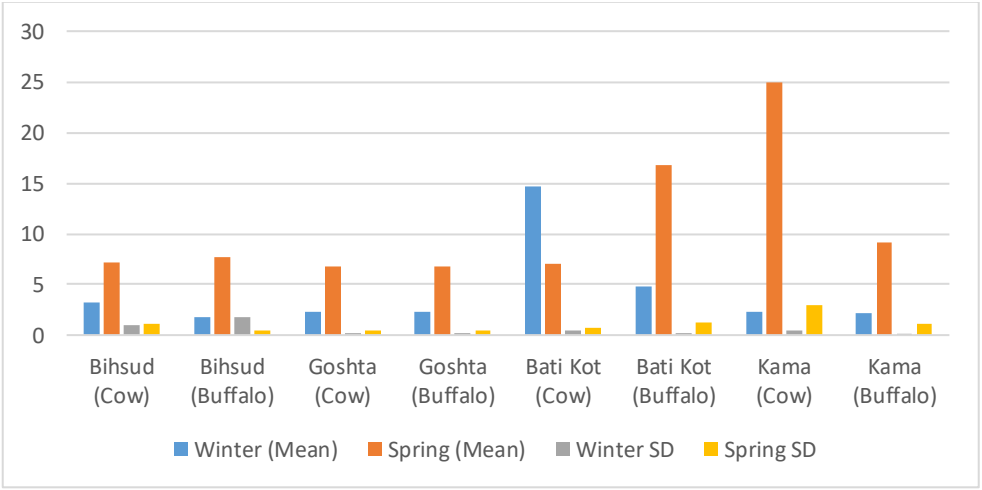


Figure 2: Seasonal Variation in Total Coliform Count (TCC) in raw milk samples across districts and animal types. Unit: CFU/mL ×10⁵±SD

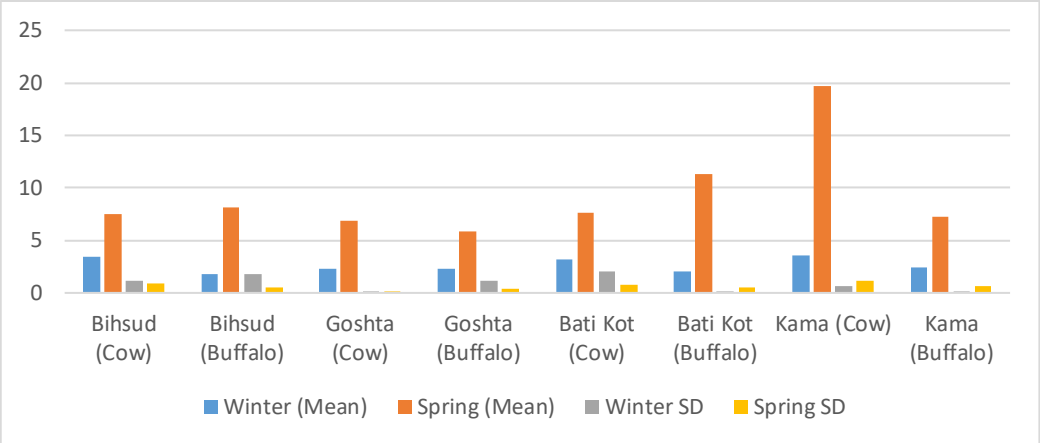


Figure 3: Seasonal Variation in E. coli Counts in raw milk samples across districts and animal types. Unit: CFU/mL $\times 10^5 \pm$SD

4 Discussion

This study assessed the microbial safety of raw milk in four districts of Nangarhar Province, with particular focus on seasonal impacts, hygiene practices, and public health implications. The primary objective was to evaluate microbial loads in cow and buffalo milk. The results demonstrate that season is a critical factor affecting milk quality, as TPC, TCC, and E. coli counts were significantly higher in spring compared to winter. This can be attributed to warmer temperatures (30–35°C in spring vs. 10–18°C in winter), which create optimal conditions for rapid bacterial proliferation (Chiarlone et al., 2025; Böhnlein et al., 2021)

Elevated TCC and E. coli counts specifically indicate inadequate hygiene and sanitation practices during milking, handling, or storage, as these organisms are widely recognized indicators of fecal contamination and poor sanitary conditions (Ndahetuye et al., 2020; Gwandu et al., 2018). Many counts exceeded internationally accepted limits (Debelu et al., 2022; Pyz-Łukasik, 2015), highlighting a tangible public health risk associated with raw milk consumption.

The observed seasonal variation is consistent with global research. Swai,(2011) reported that dairy farming conditions and milk safety are largely district-dependent, while Tassinari Ortolani et al. (2010) found higher compliance with safety standards in winter (70.4%) than in summer (51.3%), supporting the conclusion that warmer climates exacerbate microbial growth.

However, some TPC values in winter (e.g., Bihsud and Bati Kot) exceeded 5 log CFU/mL, considered unsatisfactory by (Zaki, 2025; Saidi et al., 2023), suggesting that baseline hygiene is a persistent issue, compounded by seasonal effects. This contrasts with districts employing more controlled dairy systems, where winter microbial loads typically remain within safe limits.

Theoretically, these findings reinforce the ecological principle that environmental temperature is a key driver of microbial dynamics in food products. Practically, the results emphasize the need for targeted interventions. Strategies should include improving on-farm hygiene, promoting rapid milk cooling, and educating farmers and consumers about increased microbial risks in warmer seasons. Future research should explore the specific pathogens present and the efficacy of low-cost cooling technologies to develop effective mitigation strategies.

5 Conclusions

This study provides a comprehensive assessment of the microbial quality of raw milk in Nangarhar Province, Afghanistan, focusing on Total Plate Count (TPC), Total Coliform Count (TCC), and Escherichia coli as key indicators of milk safety and hygiene. The findings demonstrate that raw milk is highly susceptible to microbial contamination, and its microbiological quality is strongly influenced by hygienic practices, handling conditions, storage duration, and ambient temperature.

Although fresh raw milk generally exhibits a low microbial load, microbial populations increase significantly with improper handling and prolonged storage at ambient temperatures. Elevated TPC values in several samples highlight deficiencies in sanitation during milking, transportation, and storage. The presence of coliforms and E. coli indicates fecal contamination and reflects poor hygienic conditions, including contaminated utensils, inadequate personal hygiene of milk handlers, unhealthy animals, and unsanitary environments.

Seasonal variation was a critical factor, with higher microbial counts observed in spring compared to winter. Warmer temperatures create favorable conditions for bacterial growth, emphasizing that time–temperature control is essential for raw milk safety, especially in districs lacking cold-chain infrastructure.

From a public health perspective, the detection of coliforms and E. coli in raw milk is concerning, as these microorganisms indicate potential foodborne pathogens. Consumption of contaminated raw milk may pose serious health risks, particularly for infants, children, the elderly, and immunocompromised individuals. Therefore, improving milk hygiene is not only a quality issue but also a vital public health priority. This research contributes novel baseline data on raw milk microbiological quality in Nangarhar Province, where scientific information is limited. The findings underscore the need for enhanced hygienic practices, farmer education, and regulatory oversight. Practical applications include training programs for milk producers and handlers, promotion of hygienic milking practices, and implementation of basic temperature control during collection and transportation.

Future research should focus on identifying specific pathogenic microorganisms, assessing antibiotic resistance patterns, and evaluating the effectiveness of interventions such as improved sanitation, pasteurization, and cold storage systems. Additionally, large-scale studies covering different provinces and seasons would further strengthen milk safety policies and support the sustainable development of the dairy industry in Afghanistan.

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5.2 Conflicts of Interest

The authors declare that there is no conflict of interes

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