

MICROBIOLOGICAL QUALITY OF RAW MILK IN THE WESTERN AND WEST CENTRAL REGIONS OF BHUTAN

KARMA T. LHADEN^{1*}, PHUNTSHO T. NORBU¹, SONAM THINLEY¹, SONAM ZANGMO¹, AND LOKAY THAPA¹

¹National Dairy Development Centre, Department of Livestock, Ministry of Agriculture and Livestock, Yusipang, Thimphu

*Author for correspondence: ktlhaden@moal.gov.bt

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Abstract: *The study aimed to evaluate the microbiological quality of raw milk in western and west-central regions of Bhutan, including Somatic Cell Counts (SCC) and the Methylene Blue Reduction Time (MBRT) test to form a basis for establishment of milk quality standards. A total of 704 individual raw milk samples and 122 bulk raw milk samples from Milk Processing Units (MPUs), Milk Collection Centres (MCCs) and milk collection sheds were tested for Total Plate Count (TPC), with random samples analyzed for SCC and MBRT. The findings indicated high microbial loads in the raw milk, with only 2.35% and 1.31% of the western region samples, and 27.33% and 21.43% of west-central region samples, complying with EC criteria for processing and consumption, respectively. Whereas, for bulk milk, none from the western region met European criteria, while 7.14% from the west-central region complied with processing criteria. The highest proportion of raw milk was observed as “bad” grade in the western region while raw milk in the west-central region was of “very bad” grade, indicating high microbial loads. For SCC, 72.09% (western) and 70.59% (west-central) of individual samples were found below 2×10^5 cells/mL, meeting EU safety standards while only 40.23% in western and 57.58% of bulk samples in west-central regions were deemed safe for direct human consumption. Thus, it is suggested that wider adoption of clean milk production practices, prompt chilling and transport below 7°C and effective pasteurization are considered prerequisites to improve the microbial quality of raw milk. Additionally, setting of milk quality standards in the country is recommended which could aid in the improvement of milk quality.*

Keywords: MBRT; Microbial quality; Quality standards; Raw milk; SCC; TPC

1. INTRODUCTION

Milk and dairy products play a vital role in providing a balanced diet for Bhutanese in addition to serving as a major source of income for dairy farmers with income dependence ranging from 50–90% across different regions (Phanchung et al. n.d). The US Department of Agriculture (USDA) in its Dietary Guidelines states the necessity of constantly including milk products in the diet, demonstrating the widespread recognition of the value of milk in a balanced diet. In Bhutan, the per capita availability of milk has increased from 113 gm/day in 2012 to 219 gm/day in 2020 (NDDC, 2023) against the FAO recommended daily allowance of 200gm.

Despite its nutritional and economic significance, milk is a highly perishable food that serves as the best medium for the growth of microorganisms, and therefore has the potential to be a source of biological and chemical hazards (Quigley et al. 2013). Although milk in the mammary gland is sterile, mammary glands of unhealthy animals, improper handling, storage and transportation can cause bacterial proliferation. Raw milk contains less than 5×10^3 cfu/ml microbes at the time of milking, but this count increases rapidly if Good Hygiene Practices (GHP) are not followed during the time of milking,

transportation and if milk is kept at ambient temperature (Kusumah et al. 2023).

The majority of dairy farmers in Bhutan rear about two milking animals in a subsistence farming system, with some taking an interest in semi-commercial ventures. In the current observed practices, sheds without assigned parlours are used for milking, and milk is delivered to roadside collection points (often simple canopies or open air) predominately in polypropylene jerry cans or bottles. Farmers either leave the milk for drivers to pick up or wait for milk collecting vans that go around designated roadside collection points for transportation to Milk Processing Units (MPUs) under non-refrigerated conditions. The milk delivered to the MPUs are subject to basic platform tests and composition analysis prior to processing into products like *datshi* (cottage cheese), butter, yogurt, paneer, or sold as unpasteurized liquid milk. These currently adopted non-standardized practices of milking, collecting, and processing can negatively impact the microbiological quality and safety of raw milk.

According to research conducted to assess the microbiological quality of milk products, pathogens such as *E. coli*, *S. aureus* and *Salmonella* were present in traditionally produced *datshi* and butter (Choki et al. 2021). Additionally, a wide range of pathogenic microorganisms of 18 different groups have been isolated from raw milk, with antimicrobial resistance observed in milk-borne pathogens (Rai et al. 2018). Markusson et al. (2021) underscores the critical importance of ensuring good microbiological quality in raw milk, as it serves as the primary raw material for producing pasteurized milk and dairy products of superior quality. Furthermore, studies conducted in one of the districts in Bhutan (Penjor & Gyeltshen 2018), as well as in other countries such as

India (Sarkar, 2016) and Nepal (Banik et al. 2014), have reported similar concerns regarding the microbiological quality of raw milk, largely attributed to inadequate hygiene practices and the absence of regulatory standards for milk and milk products. . Despite these findings, no scientific studies have been conducted in Bhutan to assess the microbiological quality of raw milk with the objective of establishing quality standards. Therefore, this study was designed to assess the raw milk microbiological quality by evaluating the Total Plate Count (TPC), Somatic Cell Count (SCC) and Methylene Blue Reduction Time (MBRT) test of raw milk in western and west-central regions of Bhutan to derive milk quality standards.

2. MATERIALS AND METHOD

2.1 Sample Collection

For sampling and analysis, Dairy Farmers' Groups (DFGs) were selected based on the number of members and the volume of milk produced. Representative samples were collected following sampling Standard Operating Procedures (SoP) in 50 ml sterile containers from the members of each selected group at their collection points. Instantly the samples were stored in a refrigerated box at less than 4°C and then transferred to National Dairy Development Centre (NDDC) for analysis.

The samples for the western region were collected from Thimphu [Kawang, Mewang, Genekha and Chang *Gewogs* (sub-district)], Chukha (Darla and Sampheling *Gewogs*) and Paro (Luni, Tsento, Doteng and Shari *Gewogs*) and from Wangduephodrang (Phobjikha, Gangtey, and Bjena *Gewogs*), Dagana (Tashiding *Gewog*) Punakha (Dzomi, Chubu, Guma, and Kabisa *Gewogs*), and Tsirang (Patshaling, Rangthaling and Tsholingkhar *Gewogs*) *dzongkhags* (districts) for the west central regions.

2.2 Sampling size and framework

2.2.1 Individual samples

Individual samples were randomly collected from 20% of the members from each DFGs over a period of one year for each region. In total, 704 individual samples were collected and analysed.

2.2.2 Bulk samples

Bulk milk samples were collected from the milk collection points/milk processing units after farmers pooled their milk into 40-liter or 20 liter cans. 50% of the total number of these cans were randomly selected for microbiological analysis over a period of one year for each region. A total of 122 bulk milk samples were collected.

2.3 Laboratory analysis of milk samples

2.3.1 Enumeration of total bacteria

The total microbial count in the raw milk was analyzed using Total Plate Count (TPC) technique, as described by Marutin and Peeler (2001) in the Bacteriological Analytical Manual (BAM). The samples were diluted using Maximum Recovery Diluent (MRD) up to 10^8 dilutions to obtain the colony count between 25-250, the serially diluted samples were then pour-plated in duplicates and after incubation at the standard time temperature combination, the plates containing 25–250 colonies were counted and the results were expressed in cfu/mL.

Additionally, MBRT test was carried out to assess the microbiological quality of milk and the Somatic cell counts in raw milk

samples were determined using the Lactoscan SCC (Lactoscan Bulgaria).

For grading of raw milk samples, the following Bureau of Indian Standards (BIS) criteria (IS-1479, Part 3, 1977) for MBRT was used in the study;

MBRT (h)	Milk grade
5 and above	Very good
3 and 4	Good
1 and 2	Fair
0.5 and below	Poor

2.4 Statistical Analysis

The data for the TPC, SCC, and MBRT were analyzed using descriptive statistics. A one-way ANOVA was performed, followed by post-hoc comparisons using Tukey HSD and Games-Howell tests to compare the TPC and SCC values among districts within the western and west-central regions, as well as between the two regions as a whole, in IBM SPSS Statistics (version 30).

3. RESULTS AND DISCUSSION

3.1 Total Plate Count of individual samples

In the western region, the mean TPC in individual raw milk samples collected from Paro, Chukha and Thimphu were 6.73×10^7 , 6.70×10^7 , and 4.28×10^7 cfu/mL, respectively. In the west-central region, the mean TPC of individual raw milk samples from Wangdue, Dagana, Punakha and Tsirang were 10.48×10^7 , 11.21×10^7 , 1.14×10^7 , and 5.91×10^7 cfu/mL, respectively (Table 1).

Table 1: TPC (cfu/mL) in individual raw milk samples in western and west-central region

Region	Districts	N	Mean \pm SE (10^7 cfu/mL)	Minimum (10^3 cfu/mL)	Maximum (10^8 cfu/mL)
Western	Paro	82	6.73 ± 1.51	18.18	4.89
	Chukha	94	6.70 ± 1.25	4.55	4.55
	Thimphu	206	4.28 ± 0.56	4.05	4.39
	Overall mean	382	5.40 ± 0.54	4.05	4.89
West-Central	Wangdue	101	10.48 ± 1.93	25.00	8.65
	Dagana	60	$11.21 \pm 3.17^*$	12.50	9.95
	Punakha	72	1.14 ± 0.53	25.00	3.05
	Tsirang	89	5.91 ± 1.68	25.00	9.35
	Overall mean	322	7.26 ± 0.99	12.50	9.95

*cfu/mL=colony forming unit per mL, N=Total number of samples, * significant difference ($p < 0.05$)

The result indicated that in the western region the mean TPC was recorded as 5.40×10^7 while in the west central region it was recorded as 7.26×10^7 cfu/mL.

There was no significant difference ($p > 0.05$) in the TPC of individual raw milk samples among districts in the western region. However, Dagana district exhibited significantly higher ($p < 0.05$) mean TPC in the individual raw milk samples compared to other districts in the west-central region due to delays in chilling. In other districts, milk samples were promptly chilled to below 7°C within 2 hours at the collection point or sheds, unlike in Dagana, where samples were collected at the MPU after farmers individually delivered milk, leading to longer exposure to ambient temperature. Cox et al. (1998) validated that faster chilling reduces microbial counts, while delays in chilling beyond 2 hours resulted in higher microbial proliferation. Additionally, there was no significant difference ($p > 0.05$) in mean TPC of individual raw milk samples between western and west-central regions.

3.2 Total Plate Count of bulk samples

The overall mean TPC in bulk raw milk in the western region was 1.98×10^8 cfu/mL, with the mean TPC of 1.24×10^8 , 3.87×10^8 , and 1.63×10^8 cfu/mL recorded for Paro, Chukha, and Thimphu, respectively.

Similarly, in the west-central region, the overall mean TPC was 6.49×10^8 cfu/mL, with the individual district's mean TPC of 6.28×10^8 , 3.56×10^8 , 1.69×10^8 , and 12.28×10^8 cfu/mL for Wangdue, Dagana, Punakha, and Tsirang respectively (Table 2).

For the bulk raw milk samples, there was no significant difference ($p > 0.05$) in the mean TPC among the districts within the western and west-central regions. However, the TPC of bulk milk sample from west-central region was significantly higher ($p < 0.05$) than the western region. Apart from factors such as poor hygiene and inadequate chilling facilities throughout the dairy value chain, the lower elevation and warmer climate of the study area in the west-central region compared to the western region, likely contributed to the higher TPC. Weerasinghe et al. (2017) also reported that bacterial count is generally higher at lower altitudes compared to higher altitudes.

3.3 Individual vs. bulk raw milk samples

In the western region, bulk raw milk samples from Chukha and Thimphu had a significantly higher ($p < 0.05$) mean TPC compared to individual raw milk samples (Table 1 and 2). However, no significant difference ($p > 0.05$) was observed in the

Table 2: TPC (cfu/mL) in bulk raw milk samples in western and west-central region

Region	Districts	N	Mean \pm SE (10^8 cfu/mL)	Minimum (10^4 cfu/mL)	Maximum (10^8 cfu/mL)
Western	Paro	15	1.24 ± 0.38	654.55	4.11
	Chukha	15	3.87 ± 2.98	30.91	45.45
	Thimphu	50	1.63 ± 0.40	95.45	10.73
	Overall mean	80	1.98 ± 0.61	30.91	45.45
West-Central	Wangdue	13	6.28 ± 2.10	100.00	25.00
	Dagana	8	3.56 ± 3.07	280.00	25.00
	Punakha	9	1.69 ± 1.46	2.50	13.30
	Tsirang	12	12.28 ± 10.30	149.00	125.00
	Overall mean	42	$6.49 \pm 3.05^*$	2.50	125.00

*cfu/mL=colony forming unit per mL, number of samples, * significant difference ($p < 0.05$)

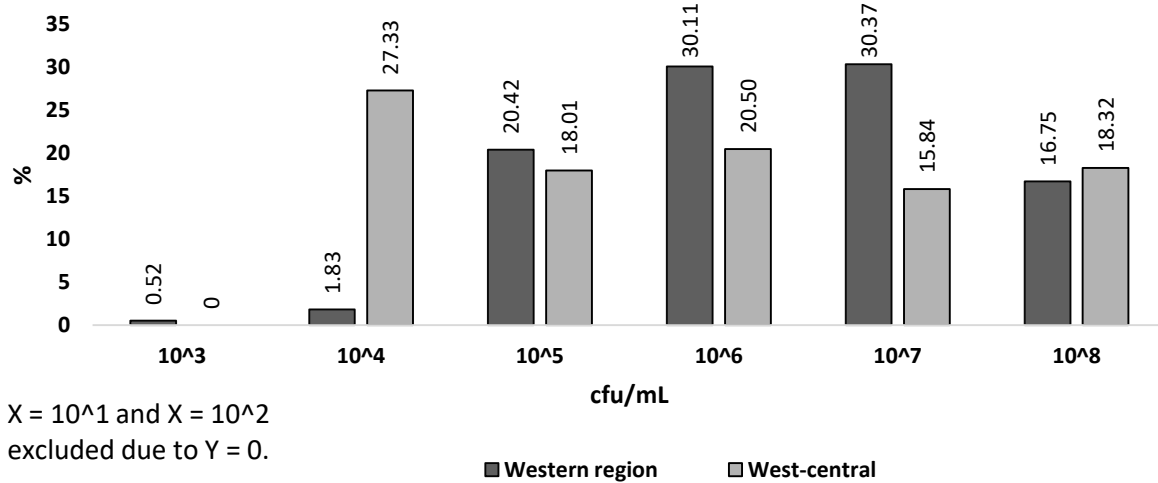


Figure 1: Presence of TPC in individual raw milk samples from the Western and West-Central region

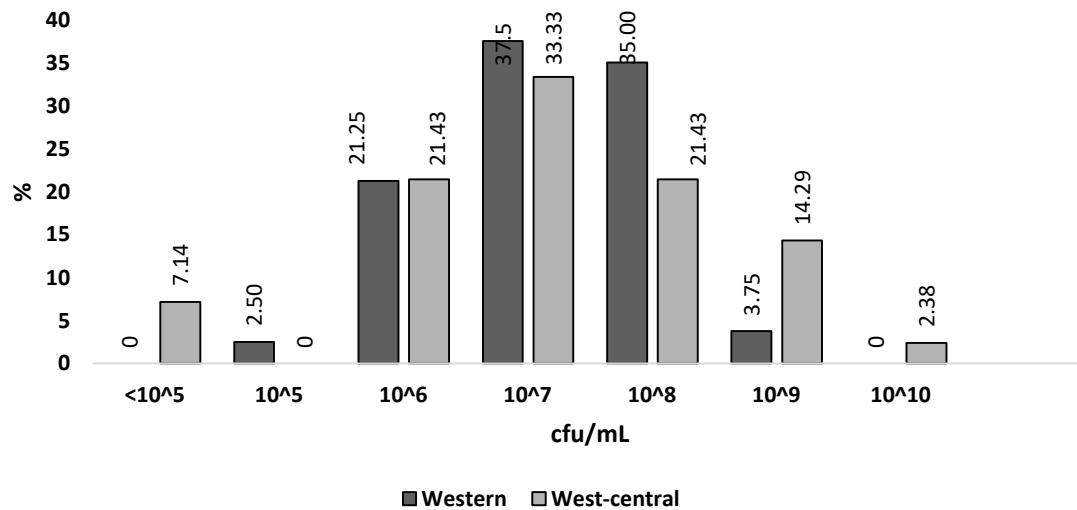


Figure 2: Presence of TPC in bulk milk samples from the Western and West-Central regions

mean TPC between bulk and individual raw milk samples in Paro. In the west-central region, bulk raw milk samples in all four districts exhibited significantly higher ($p < 0.05$) mean TPC compared to individual raw milk samples. The study conducted by Guya et al. (2019) also reported that the mean TPC was significantly ($P < 0.05$) higher at MCCs and MPUs than that at the producer's level due to further contamination of the milk during transportation, use of poorly cleaned milk containers, mixing of milk from different sources, delay in reaching out the milk to collection points, adulteration of milk with water and absence of cooling facilities. The adulteration of milk with water could be

one of the reasons for increased microbial contamination in the raw milk in these two regions.

According to the European Commission's (EC) microbiological criteria for dairy products, the TPC for raw milk intended for processing should not exceed 10^5 cfu/mL, while for raw milk intended for human consumption should not exceed 5×10^4 cfu/mL. Furthermore, high bacterial counts, exceeding 1×10^6 cfu/mL, can negatively affect product quality and safety, causing off-flavors and posing significant risks from pathogens (Murphy et al. 2016).

The assessed microbial load of raw milk is significantly high with only 2.35% and

1.31% of samples complying with the EC microbiological criteria for raw milk intended for processing and consumption respectively in the western region, while 27.33% and 21.43% of samples complied with the microbiological criteria in the west-central region. In the western region, the highest proportion of individual raw milk samples (30.37%) had TPC values in 10^7 cfu/mL while in the west-central region, 27.33% of samples recorded TPC values in 10^4 cfu/mL. Alarming, in both regions, the TPC reached as high as 10^8 cfu/mL indicating the poor quality of raw milk with the highest microbial loads being unsuitable for consumption or use in producing high-quality, value-added dairy products.

From the bulk raw milk, 7.14% of samples from the west-central region complied with the microbiological criteria set by the EC, while none of the samples from the western region could meet the criteria. The majority of samples had high microbial loads, with 37.5% and 33.33% of bulk samples in the western region and west-central regions respectively having TPC values of 10^7 cfu/mL. Additionally, 2.38% of the samples from the west central region has TPC as high as 10^{10} cfu/ml.

A high TPC in both the regions could be attributed to intrinsic factors (systemic or localized infection like mastitis) as well as extrinsic factors (contamination from faeces and poor milking hygiene). Non-hygienic conditions, such as unclean shed floors, dirty water and feed troughs, and unsanitary pre-milking practices (e.g., not washing or drying udders properly), reduce milk quality by increasing bacterial counts and milk acidity (Suranindiyah et al. 2015).

The EC standards recommend milk must be cooled immediately (within two hours from the end of milking) to not more than 8°C in

the case of daily collection or not more than 6°C if collection is not daily, during transport, the cold chain must be maintained and on arrival at the establishment, the temperature must not be more than 10°C (European Commission 2016). However, the current findings indicated that the required cold chain temperature for delivery of milk was not maintained as the recorded temperature of individual and bulk samples were between 15°C - 25°C in the western and 20°C - 30°C in the west-central regions. The spike in the TPC of raw milk could have been caused by the lack of required cold chain facilities to maintain the temperature of milk at 7°C. According to Azeze & Tera (2015), Leclair et al. (2019), and Muir et al. (1978) ambient temperature is suitable for the proliferation of microbes and deterioration of milk quality. Additionally, in the west-central region, 49% of farmers were found using PET bottles and PP cans, slightly more than 47% in the western region. These containers, which harbour microbes, likely contributed to higher TPC in raw milk. Wafula (2016) reported higher microbial contamination in plastic containers compared to aluminium and stainless-steel utensils.

The mean TPC of raw milk recorded in our study was higher than those reported in countries such as India, Bangladesh, Nepal, Malaysia, Thailand, and Indonesia (Lingathurai & Vellathurai, 2013; Khan et al. 2008; Chanda et al. 2008; Acharya et al. 2017; Chye et al. 2004; Ruangwittayanusorn et al. 2016; Fadillah et al. 2023). This can be attributed to similar challenges reported in these regions, including the lack of clean milk production practices at the farm level and inadequate chilling facilities, which contribute to

higher bacterial counts. This implies that, in terms of these practices and milk quality, the milk quality in our study is substandard when compared to that of these countries.

3.4 Methylene Blue Reduction Time (MBRT)

Based on MBRT, in the western region, Paro district has as low as 1.1% of raw milk samples categorized as “very good” grade. Majority of raw milk in three districts fall under the “fair grade”, with 49.4% in Paro, 60% in Chukha and 68.2% in Thimphu. Thimphu district also has the highest proportion of “poor” grade milk at 30.3% compared to the other two districts (Table 3).

While comparing the results from four districts in the west-central region, Punakha showed the highest proportion of raw milk classified as “very good” grade, with

56.9%. In contrast, the majority of raw milk in Wangdue, Dagana, and Tsirang fall under the “poor” grade category, with proportions of 48.5%, 52.5%, and 57.8%, respectively (Table 4).

The average temperature of raw milk in the Punakha district at the time of sampling was lower compared to other districts and this could have inhibited microbial growth. A review done by Horn et.al. (2020) highlights that lower temperature reduced the microbial growth in milk.

In the western region, 58.3% of the total samples are of “bad” grade according to Indian standards; the estimated bacterial count in the milk was 4×10^6 to 2×10^7 and such milk, without pasteurization, can remain good for only 10 hours without losing its natural physical, chemical, and

Table 3: Milk grade categorization based on MBRT in Western region

Milk Grade	Paro		Chukha		Thimphu	
	N	%	N	%	N	%
Very good	87	1.10	75	0.00	66	0.00
Good		36.80		26.70		1.50
Fair		49.40		60.00		68.20
Poor		12.60		13.30		30.30

**Note: Categorization based on BIS standard*

Table 4: Milk grade categorization based on MBRT in West-central region

Milk Grade	Wangdue		Dagana		Punakha		Tsirang	
	N	%	N	%	N	%	N	%
Very Good	101	21.80	59	5.10	72	56.90	90	13.30
Good		6.90		20.30		15.30		12.20
Fair		22.80		22.00		15.30		16.70
Poor		48.50		52.50		12.50		57.80

**Note: Categorization based on BIS standard*

organoleptic properties. Similarly, in the west-central region, 43.8% of samples are of 'very bad' grade; the milk contains a bacterial count of approximately more than 2×10^7 , and its keeping quality is less than 10 hours (Table 5). The study conducted by O'Connell et al. (2016) found that milk produced on-farm with minimal bacterial contamination can be successfully stored at 2°C and 4°C for up to 96 hours with little effect on its microbial quality.

3.5 Somatic Cell Count (SCC)

The mean SCC in individual raw milk samples in Paro, Chukha and Thimphu of western region were 7.56×10^4 , 10.93×10^4 and 24.07×10^4 cells/mL respectively. The mean SCC in Wangdue, Dagana, Punakha and Tsirang of the west-central region were 9.42×10^4 , 25.78×10^4 , 17.30×10^4 , and 18.40×10^4 cells/mL respectively. The overall mean SCC for the western region was recorded at 17.71×10^4 cells/mL and that for west-central region was 19.05×10^4 cells/mL (Table 6)

Table 5: Grading of milk quality in the western and west-central regions

Grade	Reduction time (Hours)	Approx. keeping quality/ml (Hours)	Approx. bacterial count per ml	Milk Grade (% of total samples)	
				Western	West-central
Good	5 ½ or more	40	Below 5×10^5	0.4	24.2
Fair	2 to 5 ½	30	5×10^5 to 4×10^6	23.2	12.7
Bad	20 mins to 2 hr	10	4×10^6 to 2×10^7	58.3	19.3
Very Bad	20 mins or less	Less than 10	Above 2×10^7	18	43.8

[Note: Grading as per Indian standards (IS 1479)]

Table 6: SCC (Cells/mL) in individual raw milk samples in western and west-central region

Region	Districts	N	Mean \pm SE (10^4)	Minimum (10^2)	Maximum (10^5)
Western	Paro	7	7.56 ± 4.06	0.00	2.86
	Chukha	12	10.93 ± 3.66	20.00	3.47
	Thimphu	24	24.07 ± 5.62	60.00	11.20
	Overall mean	43	17.71 ± 3.50	0.00	11.20
West-Central	Wangdue	24	9.42 ± 1.89	0.00	4.03
	Dagana	44	25.78 ± 6.93	0.00	20.51
	Punakha	10	17.30 ± 6.36	1.00	6.50
	Tsirang	74	18.40 ± 2.72	0.00	10.42
	Overall mean	152	19.05 ± 2.47	0.00	20.51

The mean SCC in bulk raw milk sample were 2.54×10^5 , 2.06×10^5 , and 2.35×10^5 cells/mL in Paro, Chukha, and Thimphu, and 1.49×10^5 , 0.94×10^5 , 2.94×10^5 , and 7.94×10^5 in Wangdue, Dagana, Punakha,

and Tsirang, respectively. In contrast to the west-central region, which has an overall mean SCC of 3.22×10^5 , the western region has a lower mean SCC of 2.34×10^5 (Table 7).

Table 7: SCC (Cells/mL) in bulk raw milk samples in western and west central region

Region	Districts	N	Mean \pm SE (10^5)	Minimum (10^3)	Maximum (10^5)
Western	Paro	21	2.54 ± 0.31	7.00	5.29
	Chukha	17	2.06 ± 0.33	25.00	4.52
	Thimphu	49	2.35 ± 0.18	5.00	5.30
	Overall mean	87	2.34 ± 0.14	5.00	5.30
West-Central	Wangdue	14	1.49 ± 0.29	4.00	3.83
	Dagana	5	0.94 ± 0.40	0.00	1.91
	Punakha	8	2.94 ± 1.07	3.00	7.98
	Tsirang	8	$7.94 \pm 2.69^*$	45.00	24.71
	Overall mean	35	$3.22 \pm 0.79^*$	0.00	24.71

*N=Total number of samples, * significant difference ($p < 0.05$)

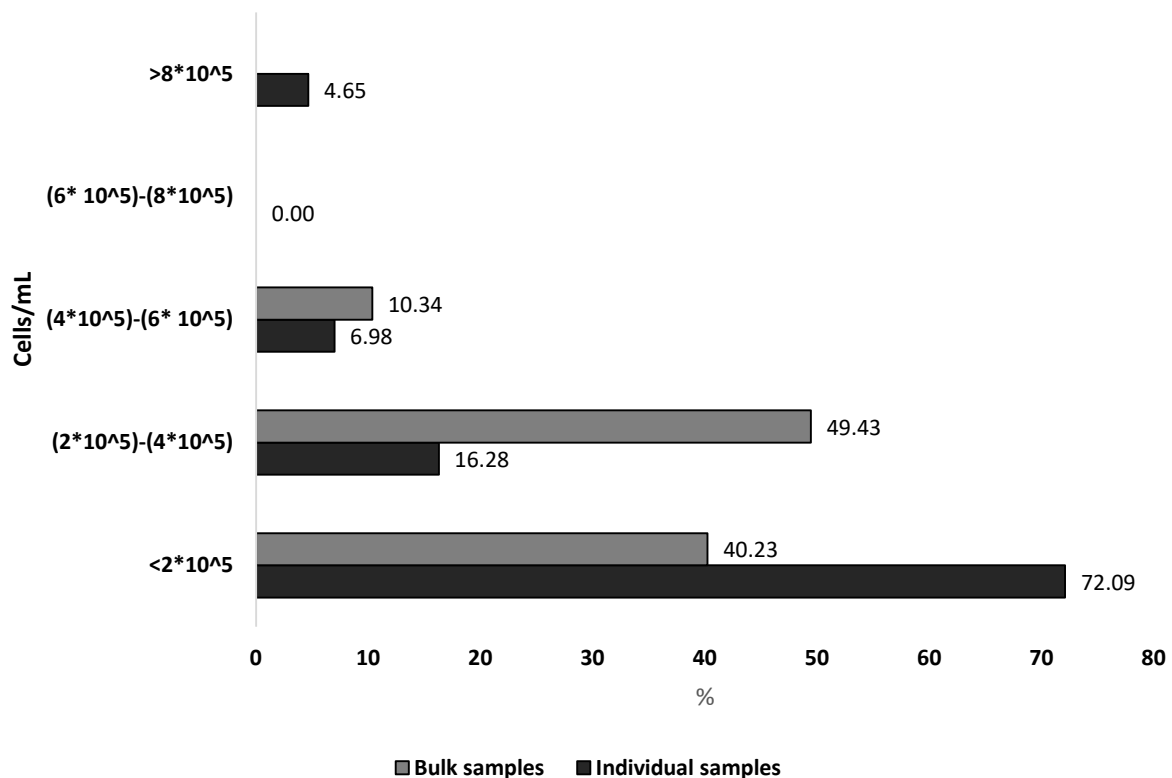


Figure 3: Presence of SCC in individual and bulk raw milk samples from the Western region

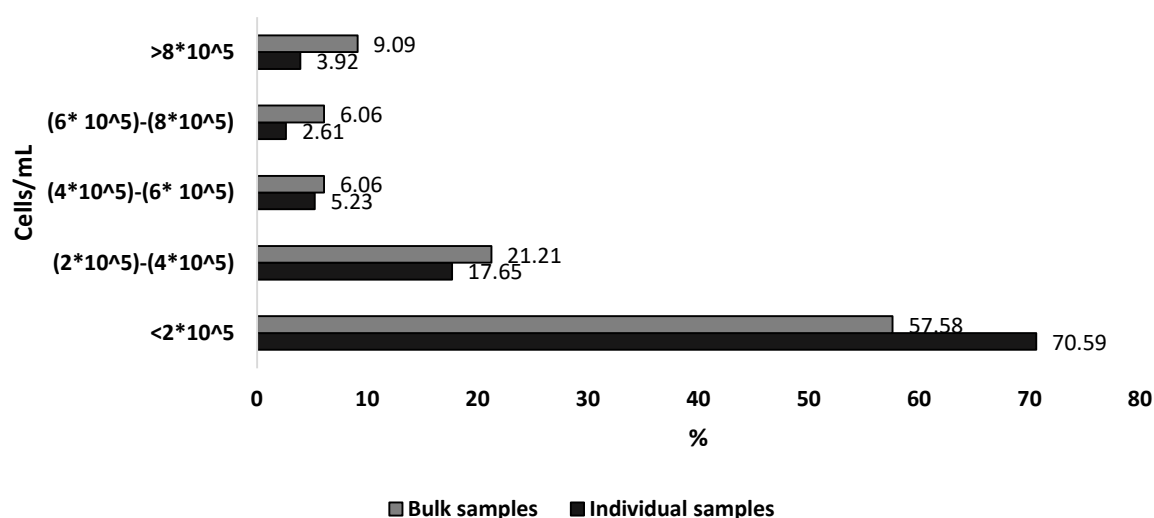


Figure 4: Presence of SCC in individual and bulk raw milk samples from the West-Central region

In the west-central region, Tsirang recorded significantly higher SCCs ($p < 0.05$) compared to the other three districts. Furthermore, the west-central region had significantly higher SCCs ($p < 0.05$) than the western region.

Among the individual raw milk samples, 72.09% from the western region and 70.59% from the west-central region had SCC less than 2×10^5 cells/mL, indicating that the milk is safe for direct human consumption and further processing according to the EC standards. However, 11.63% of samples in the western and 11.76% of samples in the west-central region had counts exceeding 4×10^5 cells/mL, which implies that the milk is unfit for direct human consumption as per the EC standards. In the case of bulk raw milk samples, 40.23% from the western region and 57.58% from the west-central region were deemed safe for direct human consumption, while 10.34% and 21.21% of bulk milk samples from the west and west-central region respectively, were found not safe for the direct human consumption. According to Holdaway et al. (1996), estimates of bulk milk somatic cell count

(BMSCC) are correlated with the prevalence of mastitis in the herd while Sprock (2024) indicated that herds with BMSCC of 400,000 cells/mL have (clinical or subclinical) mastitis infections in 40% of cows, and herds with BMSCC of 300,000 cells/mL have mastitis infections in 30% of cows. However, elevated levels of SCC in cows producing colostrum milk (early lactation) and in late lactation is also reported due to the lower milk quantity that concentrates the SCCs in milk (AHDB 2024).

According to research by Mondini et al. (2024), the most significant cause of elevated SCC is mastitis, which leads to an influx of somatic cells into raw milk as an immune response. A study by Rai et al. (2018) revealed a very high bovine mastitis prevalence rate of 20.7% for clinical mastitis and 67% for sub-clinical mastitis in Bhutan. The high SCC observed in raw milk from western and west-central regions can be attributed to mastitis, often caused by unhygienic milking practices, such as failure to use teat dips or properly clean the udder and hands, as well as poor udder health and inadequate hygienic conditions,

including dirty and wet bedding and sheds. Estimates of the number of new infections that occur in the unhygienic animal housing area range from 80 to 94 % (Costa & Reinemann 2004). The use of automatic milking equipment, teat dipping techniques, pre- and post-milking teat disinfection, and bedding type is reported to significantly influence the reduction of bulk milk SCC and bacterial counts (Jayarao et al. 2004).

This study indicates that there is some level of correlation between MBRT and TPC. Earlier studies have also shown strong correlation between MBRT and TPC (Silva et al. 2015, Prashanth and Venkatesh 2006). Additionally, Pantoja et al. (2009) reported that higher SCC and elevated milk temperatures lead to higher bacterial count. Hence, TPC, MBRT, and SCC are interrelated parameters that determine the overall microbiological quality of milk including hygiene and the extent of microbial contamination.

4. CONCLUSION

The microbiological quality of raw milk in western and west-central regions of Bhutan is currently substandard compared to international microbial criteria and standards, with high microbial counts and elevated SCC, thereby making the raw milk unsafe for consumption and unsuitable for processing into products. The TPC in the majority of the samples for both regions exceeds the international acceptable limits which could indicate non-adoption of hygienic milking practices by the dairy farmers. Thus, in order to address these challenges, it is essential for all stakeholders in the dairy value chain to adopt clean milk production practices and also ensuring that milk is chilled to below 7°C prior to processing to enhance the microbial quality of raw milk. The current findings also suggest that the MBRT test, which correlates with TPC, could serve as a cost-effective tool for assessing raw milk quality at MCCs and MPUs and devise corrective measures. Since the present

study was confined to the western and west-central regions of the country, there is need to expand research in other regions to capture a holistic picture on the overall quality of milk. Comprehensive information is essential to devise the mechanisms to improve the quality of milk and develop milk quality standards in the country.

References

- Acharya S, Bimali NK, Shrestha S, and Lekhak B. (2017). Bacterial analysis of different types of milk (pasteurized, unpasteurized and raw milk) consumed in Kathmandu Valley. *Tribhuvan University Journal of Microbiology*, 4: 32-38.
- AHDB (n.d). Somatic cell count: An indicator of milk quality. Somatic Cell Count, an indicator of milk quality. Accessed 24 September 2024.
- Azeze T, and Tera A. (2015). Safety and quality of raw cow milk collected from producers and consumers in Hawassa and Yirgalem areas, Southern Ethiopia. *Food Science and Quality Management*.
<https://www.iiste.org/Journals/index.php/FSQM/article/viewFile/26356/27001> . Accessed 4 September 2024.
- Banik SK, Das KK, and Uddin MA. (2014). Microbiological quality analysis of raw, pasteurized, UHT milk samples collected from different locations in Bangladesh. *Stamford journal of microbiology*, 4(1): 5-8.
- Cabinet Secretariat, Royal Government of Bhutan. (2024). Thirteenth Five Year Plan (2024–2029).
<https://www.pmo.gov.bt/wp-content/uploads/2019/09/13-FYP.pdf>. Accessed 21 August 2024.
- Chanda GC, Uddin GMN, Deb A, Bilkis T, Chowdhury S, and Uddin MB. (2008). Microbiological profile of the traditionally collected industrial raw milk from the milk pocket zones of Bangladesh. *Bangladesh Journal of Microbiology*, 25(1): 17-20.

- Choki K, Zangmo S, and Norbu PT. (2021). Microbial quality of traditionally produced butter and cheese (datshi). *Bhutan Journal of Animal Science*, 5(1): 1-7.
- Chye FY, Abdullah A, and Ayob MK. (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food microbiology*, 21(5): 535-541.
- Costa DA, and Reinemann DJ. (2004). The purpose of the milking routine and comparative physiology of milk removal. Paper presented at the 2004 meeting of the National Mastitis Council. University of Wisconsin-Madison Milking Research and Instruction Lab.
- Cox IM, Adapa S, and Schmidt KA. (1998). Cooling rate and storage temperature affect bacterial counts in raw milk (1998). *Kansas Agricultural Experiment Station Research Reports*, 2: 36-39.
- De Silva SASD, Kanugala KANP, and Weerakkody NS. (2015). Microbiological quality of raw milk and effect on quality by implementing good management practices. <https://www.sciencedirect.com/science/article/pii/S2211601X16000201>. Accessed 12 September 2024.
- European Commission. (2016). European guide for good hygiene practices in the production of artisanal cheese and dairy products: Target: Farmhouse and artisan producers (Revised version of 20th December 2016). Office of the European Union. https://food.ec.europa.eu/system/files/2017-12/biosafety_fh_guidance_artisanal-cheese-and-dairy-products_en.pdf. Accessed 1 October 2024.
- Fadillah A, van den Borne BH, Poetri ON, Hogeveen H, Slijper T, Pisestyani H, and Schukken YH. (2023). Evaluation of factors associated with bulk milk somatic cell count and total plate count in Indonesian smallholder dairy farms. *Frontiers in Veterinary Science*, 10: 1280264.
- Guya ME, Adugna MM, and Mammed Y. (2019). Milk production, marketing, and quality in Meta District of Eastern Hararghe Zone, Ethiopia. *Journal of Agricultural Science*, 11(5), 535.
- Holdaway RJ, Holmes CW, Steffert UA. (1996). comparison of indirect methods for diagnosis of subclinical intramammary infection in lactating dairy cows Part 3. *Australian Journal of Dairy Technology*, 51:79-82.
- Horn B, Pattis I, and Soboleva T. (2020). Growth of microorganisms in raw milk: Evaluating the effect of chiller failure (New Zealand Food Safety Technical Report No. 2020/04). New Zealand Food Safety.
- Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR, and Hegde NV. (2004). Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. *Journal of Dairy Science*, 87(10): 3561–3573. [https://doi.org/10.3168/jds.S0022-0302\(04\)73493-1](https://doi.org/10.3168/jds.S0022-0302(04)73493-1).
- Khan MTG, Zinnah MA, Siddique MP, Rashid MHA, Islam MA, and Choudhury KA. (2008). Physical and microbial qualities of raw milk collected from Bangladesh agricultural university dairy farm and the surrounding villages. *Bangladesh Journal of Veterinary Medicine*, 6(2): 217-221.
- Kusumah NAF, Widjiati W, Puspitasari Y, Tyasningsih W, Wardhana DK, Permatasari DA, and Luqman EM. (2023). Comparison of total number of bacteria in raw milk Friesian Holstein cow based on milking techniques using total plate count (TPC) test. *International Journal of Scientific Advances*, 4(1): 37-40.
- Leclair RM, McLean SK, Dunn LA, Meyer D, and Palombo EA. (2019). Investigating the effects of time and temperature on the growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in raw cow's milk based on simulated consumer food handling practices. *International Journal of Environmental Research and Public Health*, 16(15): 2691.
- Lingathurai S, and Vellathurai P. (2013). Bacteriological quality and safety of raw cow milk in Madurai (South India). *Bangladesh Journal of Scientific and Industrial Research*, 48(2): 109-114.
- Marutin L, and Peeler JT. (2001). BAM Chapter 3: Aerobic plate count. *Laboratory Methods (Food)*. Food and Drug Ministration (FDA). <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count#conventional>. Accessed 13 August 2024.
- Mondini S, Gislon G, Zucali M, Sandrucci A, Tamburini A, and Bava L. (2024). Factors influencing somatic cell count and leukocyte composition in cow milk: A

- field study.
<https://doi.org/10.3168/jds.2024-25357>.
- Muir DD, Kelly ME, and Phillips JD. (1978). The effect of storage temperature on bacterial growth and lipolysis in raw milk. *International Journal of Dairy Technology*, 31(4): 203-208.
- Murphy SC, Martin NH, Barbano DM, and Wiedmann M. (2016). Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? *Journal of Dairy Science*, 99(12): 10128–10149. <https://doi.org/10.3168/jds.2016-11172>.
- O'Connell A, Ruegg PL, Jordan K, O'Brien B, and Gleeson D. (2016). The effect of storage temperature and duration on the microbial quality of bulk tank milk. *Journal of Dairy Science*, 99(5): 3367-3374.
- Pantoja JCF, Reinemann DJ, and Ruegg PL. (2009). Associations among milk quality indicators in raw bulk milk. *Journal of Dairy Science*, 92(10): 4978–4987. https://www.sciencedirect.com/science/article/pii/S002203020970829X?ref=pdf_download&fr=RR-2&rr=8f207ecf2bbccdde. Accessed 5 November 2024.
- Penjor T, and Gyeltshen T. (2018). Microbial load in local and imported raw milk. *Bhutan Journal of Animal Science*, 2(1): 112-114.
- Phanchung, Dorji P, Sonam T, and Pelden K. (n.d.). Smallholder dairy farming in Bhutan: Characteristics, constraints, and development opportunities. Natural Resources Training Institute, Lobesa, Wangdue Phodrang, Bhutan.
- Prashanth B, and Venkatesh KV. (2006). Quantification of metabolically active biomass using Methylene blue dye reduction test, measurement of CFU in about 200s. *Journal of Microbiological Methods*, 65: 107-116.
- Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF, and Cotter PD. (2013). The complex microbiota of raw milk. *FEMS microbiology reviews*, 37(5): 664-698.
- Rai SBC, Sharma PM, Tenzinla, Mata P, Tshomo P, and Gurung BB. (2018). Microbiological quality of raw milk in Bhutan. *Bhutan Journal of Animal Science*, 2(1): 1-10.
- Reddy IS, and Puniya AK. (n.d.). Introductory Dairy Microbiology. Department of Dairy Microbiology, SVVU, Tirupati, & Dairy Microbiology Division, NDRI, Karnal.
- Ruangwittayanusorn K, Promket D, and Chantiratikul A. (2016). Monitoring the hygiene of raw milk from farms to milk retailers. *Agriculture and Agricultural Science Procedia*, 11: 95-99.
- Sarkar S. (2016). Microbiological safety concerns of raw milk. *Journal of Food Nutrition and Dietetics*, 1(2):1-7
- Sprock M. (2024). Bulk milk somatic cell count and cow health. *West Coast Vets*. <https://www.wcvets.co.nz/blog/post/125576/bulk-milk-somatic-cell-count-and-cow-health/>. Accessed 22 October 2024.
- Weerasinghe WPCG, Hettiarachi S, and Jayarathne MPK. (2017). Factors affecting the quality of raw milk: Effect of time taken for transportation and practices at field level in small farms in Sri Lanka. *Research & Reviews: Journal of Food and Dairy Technology*.