S. L. Veterinary Journal 72(1)
Original Research Article
DOI: https://doi.org/10.4038/slvj.v72i1.92

ASSESSMENT OF TOTAL BACTERIAL COUNT IN MILK COLLECTION CHAIN IN DIFFERENT CLIMATIC ZONES OF SRI LANKA

L. M. P. Wijemanne[1*] K. Suthaharan[2] S. De Alwis[1] I. Vithanage[1]
[1]*Dairy Technology Laboratory, Veterinary Research Institute, Gannoruwa, Peradeniya 24000*
[2]*Veterinary Investigation Centre, Vavuniya 43000, Sri Lanka*

SUMMARY: Cow milk is initially contaminated on the inner surface of the teat canal. Microbes from teat surfaces, milkers' hands, equipment, poor hygiene and unhygienic environments contribute to raw milk contamination on farms and along the milk collection chain, leading to high bacterial counts that compromise milk quality. Total Bacterial Count (TBC) is a key indicator of raw milk quality, relevant to processors, consumers, and public health decision making. This study aimed to assess TBC along the milk collection chain, including farms, milk collecting points (MCP), milk chilling centres (MCC), and a milk processing plant (MPP) across different climatic zones in Sri Lanka from January to December 2024. A total of 353 raw milk samples were collected from farm cans, 40 L cans, chilling tanks, bowsers, and storage tanks, and tested by Total Plate Count (TPC). Samples included 93 (farm), 150 (MCP), 46 (MCC), and 64 (MPP). Median TBC values were 4.40×10^6, 4.80×10^7, 9.35×10^7, and 6.00×10^8 cfu/ml, respectively, while mean values were 2.11×10^8, 3.79×10^8, 6.11×10^8, and 1.27×10^9 cfu/ml. TBC differed significantly among collection points ($p<0.05$), with Tukey's HSD test identifying milk at the MPP as most contaminated. A substantial increase was observed from MCP to MPP. No statistically significant relationship was found between TBC and milk temperature, likely due to fluctuations during transport. Titratable acidity showed a weak, non-significant positive correlation with TBC. This study demonstrates progressive bacterial contamination along the collection chain, with the highest levels at the processing plant. Adoption of good hygienic practices at farm and collection levels is essential to ensure milk safety, improve product quality, and protect public health.

KEYWORDS: Milk quality, Total Bacterial Count, Milk Collecting Centres, Milk Chilling Centres, Milk Processing Plants, Sri Lanka

INTRODUCTION

Cow's milk is a nutritionally important food and a major source of essential nutrients for humans. In healthy animals, milk is secreted as a sterile fluid in the alveoli of the udder; however, it may become contaminated initially at the inner surface of the milk cistern and teat canal. Microorganisms present on the teat surface, milkers' hands, milking utensils, and the farm environment are common sources of microbial contamination. Furthermore, inadequate hygienic practices during milking, collection, storage, and transport exacerbate contamination, as milk provides an ideal medium for bacterial proliferation. High bacterial loads, including spoilage organisms and potential human pathogens, compromise the safety and quality of raw milk and its products, posing risks to public health. In addition, intentional adulteration practices such as dilution or the use of additives to mask microbial spoilage further reduce milk quality. The production of high quality dairy products is therefore dependent on the microbial quality of raw milk.

Total Bacterial Count (TBC), commonly assessed using the Total Plate Count (TPC), is widely used as an indicator of raw milk quality. Regulatory standards for TBC vary internationally: the Sri Lanka Standards Institute (SLSI) has proposed a limit of 3×10^5 cfu/ml, while India's Food Safety and Standards Regulation (FSSR, 2006) set the limit at 2×10^5 cfu/ml, and the European Union Regulation 605/2010 specifies 1×10^5 cfu/ml (European Union Regulation605/2010).

In Sri Lanka, only a limited number of studies have assessed TBC along the milk collection chain. For example, Ubeyratne *et al*. (2014) reported mean total viable counts of 10^6 cfu/ml in raw milk at primary production in the Central Province. Similarly, Senarath *et al*. (2017) observed elevated Standard Plate Counts (SPC) in samples from chilling centres, transportation vessels, and farms in Anuradhapura District, while De Silva *et al.* (2015) reported TPC levels of 10^6–10^7 cfu/ml in chilling centres in Kurunegala District. However, these studies were limited to selected geographic areas

Corresponding Author: L. M. P. Wijemanne, E-mail: lmpwlb@gmail.com

and specific objectives, and thus are insufficient to establish a national profile of raw milk quality.

A comprehensive understanding of TBC across the milk collection chain in different climatic zones is essential for ensuring milk safety, improving compliance with food safety standards, and supporting the development of effective interventions. Therefore, the objective of the present study was to evaluate the total bacterial count in raw milk collected from dairy farms, milk collecting points, chilling centres, and processing plants in Sri Lanka.

MATERIALS AND METHODS

The study was conducted from January to December 2024. Raw milk samples were collected across the milk collection chain, including dairy farms, milk collecting points (MCPs), milk chilling centres (MCCs), and milk processing plants (MPPs). They were collected aseptically in sterile sample bottles, transported at 4 °C, and analysed on the same day. Samples from farms and MCPs were analysed at Veterinary Investigation Centre laboratories in 10 districts, while those from MCCs and MPPs were tested at the Dairy Technology Laboratory, Veterinary Research Institute. Samples received at MPPs during the night were frozen and tested the following day.

The total bacterial load was estimated using the standard microbiological method, Total Plate Count (TPC), as described by Emma *et al*. (2020). Milk samples were thoroughly mixed, and ten-fold serial dilutions were prepared by inoculating 1 ml of milk into 9 ml of 0.1% sterile peptone water. Aliquots of 0.1 ml from appropriate dilutions were spread-plated in duplicate on nutrient agar and incubated at 37 °C for 48 hours to enumerate total aerobic mesophilic bacteria, including spoilage organisms. Plates yielding 30–300 colonies were selected for enumeration, and results were expressed as colony forming units (cfu) per ml of milk using the following formula:

$$\text{Colony count (cfu/ml)} = \frac{\text{Total number of colonies} \times \text{Dilution factor}}{\text{Volume of initial sample}}$$

Titratable acidity of raw milk was determined using phenolphthalein as an indicator. A 9 ml aliquot of milk was titrated against 0.1 N sodium hydroxide solution until a faint pink endpoint was observed. Titratable acidity (%) was calculated as:

$$\text{Titratable acidity (\%)} = \text{Volume of 0.1 N NaOH (ml)} \times 0.1$$

During sampling, ancillary data including ambient temperature and transport duration were also recorded.

For statistical analysis, descriptive statistics were generated using Microsoft Excel. Further analyses, including one-way ANOVA, correlation coefficients, and Tukey's HSD test, were performed using SPSS (version 29).

RESULTS

A total of 353 raw milk samples were collected from 18 districts across Sri Lanka. Of these, 243 samples were obtained from dairy farms and Milk Collecting Points (MCPs) in nine districts (Colombo, Kalutara, Kandy, Galle, Vavuniya, Trincomalee, Badulla, Ratnapura, and Anuradhapura). The remaining 110 samples originated from Milk Chilling Centres (MCCs) and Milk Processing Plants (MPPs) located in 13 collection sites across 15 districts. The number of samples collected from farms, MCPs, MCCs, and MPPs was 93, 150, 46, and 64, respectively (Table 1).

Table 1. Descriptive statistics of the Total Bacterial Count in farms, Milk Collecting Points (MCP), Milk Chilling Centres (MCC) and Milk processing Plants (MPP)

Location sample collected	total No. of raw milk samples	Rejected samples (TBC more than 10^{10}	No of samples analysed	Median TBC cfu/ml	Mean TBC cfu/ml
Farm	93	20	73	4.40×10^{6}	2.11×10^{8}
MCP	150	32	118	4.80×10^{7}	3.79×10^{8}
MCC	46	4	42	9.35×10^{7}	6.11×10^{8}
MPP	64	9	55	6.00×10^{8}	1.27×10^{9}
Total	353	65	288	1.86×10^{8}	6.18×10^{8}

Samples with total bacterial counts (TBC) $\geq 10^{10}$ cfu/ml were excluded from analysis, accounting for 18% of total samples, primarily those from farms and MCPs. The median TBC values were 4.40×10^{6} cfu/ml (farm), 4.80×10^{7} cfu/ml (MCP), 9.35×10^{7} cfu/ml (MCC), and 6.00×10^{8} cfu/ml (MPP) (Table 1). The mean TBC values were 2.11×10^{8}, 3.79×10^{8}, 6.11×10^{8}, and 1.27×10^{9} cfu/ml for farm, MCP, MCC, and MPP samples, respectively. The overall mean TBC across the milk collection chain was 6.18×10^{8} cfu/ml. A gradual increase in bacterial load was observed from farms to MPPs (Table 2).

Table 2. No. of samples in different levels of Total Bacterial Count (TBC) in the milk collection chain

TBC	No. of samples			
	Farm	MCP	MCC	MPP
1×10^4	6	0	0	0
1×10^5	15	3	2	2
1×10^6	26	31	4	3
1×10^7	14	40	15	10
1×10^8	9	30	12	21
1×10^9	3	13	9	19
1×10^{10}	20	33	4	9
Total	93	150	46	64

Analysis of variance (ANOVA) indicated statistically significant differences in TBC among the four collection points (farm, MCP, MCC, and MPP) ($p = 0.000259$). Tukey's HSD post-hoc test revealed that milk received by MPPs had the highest contamination level (p-adj = 0.0000822). A marginally significant difference was observed between farms and MPPs (p-adj = 0.055), whereas other pairwise comparisons were not statistically significant (p-adj > 0.05) (Figure 1). These findings indicate a substantial and progressive increase in microbial contamination along the milk collection chain.

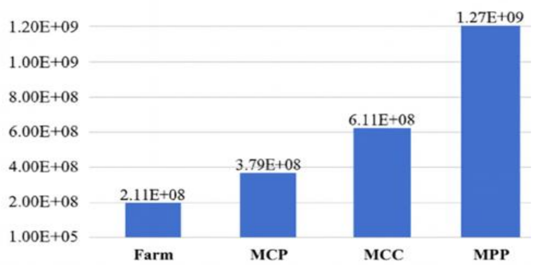

Figure 1. Mean of the TPC in different locations in the milk collection chain

Temperature, Titratable Acidity, and Handling Conditions

The mean milk temperatures recorded at the time of collection were 29°C (farm), 29°C (MCP), 23°C (MCC), and 12°C (MPP). The temperature of milk samples from farms ranged between 2°C and 37°C, while those from MCPs ranged from 15°C to 33°C. Approximately 20% of farmers reported freezing or chilling the evening milk at home, with most mixing it with freshly drawn milk the following morning. The temperature of milk samples varied considerably across districts, independent of ambient conditions; therefore, inter-district comparisons were not performed.

At MPPs, temperature data were available for 50% of samples. Among these, 27% were frozen at collection, while the remainder lacked temperature records. Correlation analysis showed no significant relationship between TBC and milk temperature at the farm level ($r = –0.478$, $p = 0.162$). A weak but statistically significant positive correlation was observed at MCPs ($r = 0.162$, $p = 0.048$). Correlations at MCCs ($r = 0.139$, $p = 0.355$) and MPPs ($r = –0.076$, $p = 0.550$) were weak and not statistically significant.

Titratable acidity, measured in 110 milk samples from MCCs and MPPs, exhibited weak positive but statistically insignificant correlations with TBC (MCC: $r = 0.214$, $p = 0.153$; MPP: $r = 0.082$, $p = 0.519$). Notably, 70% of MCC samples and 85% of MPP samples showed acidity levels below 0.17%, within the acceptable limit for raw milk as per the Sri Lanka Standards Institute (SLSI draft).

Adulteration testing identified two noncompliant samples, one from an MCC (formalin) and one from an MPP (sodium bicarbonate). The average time between milking and sampling at farms was approximately 1.5 hours. The duration between farm and MCP ranged from 15 minutes to 3 hours. From MCP to MCC, 58% of samples arrived within 1–5 hours, 28% within 1 hour, and 14% after more than 5 hours. Chilling commenced upon arrival at MCCs and required an average of 2 hours to reach 4°C. Milk transported by bowsers to MPPs took between 0.5 and 6 hours, with an average transit duration of 3 hours.

DISCUSSION

This study was conducted as a baseline investigation to determine the average total bacterial count (TBC) in raw milk collected from various regions of Sri Lanka. Samples were received from 18 districts, with the main study area encompassing ten districts representing the dry, intermediate, and wet climatic zones. Therefore, the findings provide an overview of the average TBC in raw milk across the principal climatic regions of the country.

The culture plates were incubated at 37 °C for 48 hours to determine total plate counts (TPC), with the intention of including both spoilage and potentially pathogenic bacteria in the enumeration. According to SLS 516 Part 1: Section 1, aerobic plate counts are determined by incubating plates at 25–40 °C for 48 hours, while ISO 4833-1:2013 recommends incubation at 30 °C for 72 hours for mesophilic bacteria. Similarly, Bureau of Indian Standards (IS 1479 Part 1:1960) and Food Safety and Standards Authority of India (FSSAI) specify incubation at 37 °C for 48 hours. The draft standard for TPC in raw milk in Sri Lanka is 3×10^5 cfu/mL; however, in this study, the median TBC values ranged from 10^6 to

10^8 cfu/mL at different points in the milk collection chain.

The median TBC values at the farm, milk collecting point (MCP), milk chilling centre (MCC), and milk processing plant (MPP) were 10^6, 10^7, 10^7, and 10^8 cfu/mL respectively, while the corresponding mean values were 10^8, 10^8, 10^8, and 10^9 cfu/mL (Table 1). This pattern indicates a right-skewed distribution, suggesting that median values better represent the typical microbial quality of raw milk, whereas mean values are influenced by outliers arising from heavily contaminated samples. Samples with extremely high TBCs ($\geq 10^{10}$ cfu/mL) were excluded to minimise the impact of outliers due to possible contamination or laboratory error.

ANOVA analysis revealed significant differences in TBC among the four collection points ($p = 0.000259$), indicating an overall increase in bacterial load along the milk collection chain. Tukey's HSD post-hoc test showed a statistically significant increase in TBC between MCP and MPP (p-adjusted = 0.0000822), signifying that the most substantial bacterial growth occurred during transportation between these points. The difference between the farm and MPP was marginally significant (p-adjusted = 0.0550), while other comparisons were not significant ($p > 0.05$). These findings suggest that although bacterial contamination originates at the farm level, significant bacterial multiplication occurs during milk handling and transport.

At the farm level, initial bacterial contamination was already high (10^6 cfu/mL), emphasising the importance of hygienic milking practices. Once contaminated milk enters the collection chain, favourable environmental factors such as time and temperature further enhance bacterial proliferation. Thus, improving on-farm hygiene and implementing the principles of "clean milk production" are critical to reducing initial microbial load. Similar findings have been reported in the Anuradhapura District, where microbial contamination was observed at every stage of milk handling, beginning at the farm level (Senarath *et al.*, 2017). Given that this study included samples from 18 districts, the results reflect a realistic estimate of TBC at the national level.

The temperature of milk during transportation ranged from 29 °C to 12 °C, with extremes of 37 °C and 2 °C observed at the farm level and 33 °C to 15 °C at MCPs. This temperature range permits the growth of both mesophilic and psychrotrophic bacteria. A significant relationship was observed between TBC and temperature at MCPs, although the effect was less pronounced at other points. Field observations revealed that farmers often mix refrigerated or frozen evening milk with freshly drawn morning milk before transport, exposing milk to fluctuating temperatures that favour bacterial growth. Rapid cooling of milk to 4 °C within 30 minutes can reduce bacterial growth by 50% compared to cooling after 2 hours (Cox *et al.*, 1998); however, this standard was rarely achieved in practice. Although freshly drawn milk is nearly sterile, psychrotrophic populations develop during storage even under refrigeration (Oliveira *et al.*, 2015). Therefore, repeated temperature fluctuations at the farm level substantially contribute to bacterial multiplication.

Milk cans are generally transported from MCPs to MCCs between 9 and 14 hours, often exposed to direct sunlight. Only 28% of the cans reached MCCs within one hour, mostly those delivered directly from farms. Although chilling tanks at MCCs are set to 4 °C, milk temperature fluctuates because milk is added in batches at ambient temperatures, delaying uniform cooling. In most centres, milk takes over 2 hours to reach 4 °C, during which microbial proliferation occurs (Cox *et al.*, 1998). Residual milk occasionally stored overnight at 4 °C further supports psychrotrophic growth, leading to higher TBCs at the processing plants. At MPPs, 50% of samples had an average temperature of 1 °C, with a minimum of 4 °C and a maximum of 24 °C. These variations indicate that milk undergoes temperature fluctuations during transportation and storage, promoting further bacterial growth. Transit from MCCs to MPPs can take from 30 minutes to more than 6 hours, during which milk temperature may rise to an average of 12 °C, enhancing microbial activity despite initial chilling.

Although temperature is a major determinant of bacterial growth, the correlation between TBC and milk temperature was not statistically significant in this study. This is likely due to the confounding effect of diverse bacterial species and previous temperature exposures before sampling. Moreover, unhygienic handling practices, contaminated equipment, and unclean transport vehicles also contribute to elevated bacterial loads.

The majority of milk samples showed acceptable levels of titratable acidity (0.17) in MCCs and MPPs in 70% and 85% of samples, respectively. Although increasing bacterial counts generally elevate acidity (Schmidt *et al.*, 1996), the correlation between TBC and titratable acidity was weak and statistically insignificant in this study (MCC: $r = 0.214$, $p = 0.153$; MPP: $r = 0.082$, $p = 0.519$). This may be attributed to the presence of non-acid-producing psychrotrophs, the intrinsic buffering capacity of milk, and variations in storage and enzymatic activity.

CONCLUSION

The total bacterial count differed significantly among the farm, milk collecting point (MCP), milk chilling centre (MCC), and milk processing plant (MPP), confirming that microbial contamination and bacterial proliferation occur at every stage of the milk collection chain. A marked increase in bacterial load between the MCP and MPP highlights inadequate hygienic handling and ineffective temperature control during milk transportation. The consistently high TBCs observed in raw milk received by MPPs indicate that substandard quality milk is frequently used for processing in Sri Lanka. Furthermore, the elevated bacterial counts at the farm level emphasise the importance of implementing clean milk production practices to minimise initial microbial contamination. The observed fluctuations in milk temperature and delays in cooling often exceeding the recommended 2 hours to reach 4–6 °C, further contribute to bacterial growth along the chain. Although chilling facilities are available at certain collection points, poor maintenance of the cold chain facilitates the multiplication of diverse bacterial populations in raw milk. Strengthening on-farm hygiene, ensuring rapid and consistent cooling, and maintaining the cold chain throughout transportation are essential measures to improve the microbiological quality of raw milk used for processing in the country.

ACKNOWLEDGEMENT

The initial encouragement of the Director, Veterinary Research Institute, and support extended throughout this study by the Director, Animal Health and Dr. Uditha Hettigamage, Animal Health Division, Department of Animal Production and Health are highly appreciated. Similarly, the Veterinary Investigation Officers, Dr. A. V. Vivekanandan, Veterinary Investigation Centre, Trincomalee, Dr. D. M. Gunathilaka, Veterinary Investigation Centre, Galle, Dr. Samanthi Rathnasiri, Veterinary Investigation Centre, Homagama, Dr. P. N. C. Fernando, Veterinary Investigation Centre, Anuradhapura, Dr. D. P. H Fernando, Veterinary Investigation Centre, Kalutara, Dr. K. V. I. N. Kumaravithana, Veterinary Investigation Centre, Badulla, Dr. A. R. Gangani, Veterinary Investigation Centre, Kundasale, Milk processing companies and their staff and, Dairy Technology Laboratory staff, Gannoruwa are gratefully acknowledged for their generous coorporation.

REFERENCES

Cox, I.M., Adapa, S., and Schmidt, Karen A. (1998). Cooling rate and storage temperature affect bacterial counts in raw milk. Kansas Agricultural Experiment Station Research Reports: Vol. 0: Iss. 2. https://doi.org/10.4148/2378-5977.3237

De Silva, S.A.S.D., Kanugalab, K.A.N.P., Weerakkody, N.S. (2015). Microbiological quality of raw milk and effect on quality by implementing good management practices. International Conference of Sabaragamuwa University of Sri Lanka. Procedia Food Science **6** (2016) 92 – 96. www.sciencedirect.com.

Emma, M., Geremew, B., Dereje, F. (2020). Total bacterial count of raw milk in selected dairy farms in Nekemta town. *World Journal of Dairy & Food Sciences* **15** (1): 62-69. http://www.researchgate.net.

European Union (EU) Regulation No 605/2010. Commission *Regulation* (*EU*) *No 605/2010* of 2 Jul. *https://eur-lex.europa.eu › eli › reg › eng*

Food Safety and Standard Authority of India (FSSAI). https://fssai.gov.in › cms › food-safety-and-standards-reg...Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011 ... Regulations, 2016. Regulation Pdf size:(1.11 MB).

IS 1479-1 (1960): Methods of test for dairy industry, Part 1: Rapid examination of milk [FAD 19: Dairy Products and Equipment. https://law.resource.org › pub › bis › is.1479.1.19...

International Standards ISO-4833-1. https://cdn.standards.iteh.ai › samples › ISO-4833-...PDF Sep 1, 2013 — ISO 2013.

Oliveira, I. B. G., Favarin, L., Luchese, H. R., McIntosh. (2015). Psychrotrophic bacteria in milk: how much do we really know? *Brazilian Journal of Microbiology* **46** (2):313–321.

Senarath, H. M. I. G. A. M. K., Adikari, A. M. J. B. (2017) Microbiological Quality of Raw Milk at Selected Chilling Centers in Anuradhapura District of Sri Lanka. *International Journal of Scientific and Research Publications*, **7** (11): 328. ISSN 2250-3153.

Schmidt, K., Stupar, J., Shirley, J., Adapa, S. (1996). Factors affecting titratable acidity in raw milk.**10** (2): 340 [online] available https://newprairiepress.org/kaesrr/vol0/iss2/340.

Ubeyratne, J.K.H., Jayaweera, M.D.N., Mangalika, U.L.P. (2014). Sri Lanka Microbial Quality, Composition and Keeping Quality of Raw and Heat-Treated Liquid Milk in Central Province of Sri Lanka. *Journal of the Commonwealth Veterinary Association.* **30** (1): 21-25.