

# Microbiological Quality of Raw Milk at Selected Chilling Centers in Anuradhapura District of Sri Lanka.

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## Abstract

Milk easily undergoes microbial contamination and spoilage because it is a rich biological fluid which contains all necessary nutrients for animals. This study was carried out to evaluate the microbiological quality and safety of cow milk at different sampling points; selected chilling centers, milk transportation vessels and farmers in Anuradhapura district of Sri Lanka. A total of 11 chilling centers were selected for the study and classified them as low and high risk centers. Total 80 samples were tested for total bacterial count/standard plate count (SPC) and total coliform count (TCC) and analyzed by using Poisson Regression in GENMOD Procedure in SAS. Descriptive statistics of standard plate count and total coliform count data were obtained using Minitab 17. Results revealed that higher microbial counts for SPC and TCC were observed in chilling centers, transportation vessels and farmers with compared to the standards. The SPC and TCC showed a significant difference ( $p < 0.05$ ) between low and high risk chilling centers, low and high risk farmers, chilling centers and transportation vessels and chilling centers and farmers. In conclusions, microbial contamination occurs at every point of milk handling as expected. The contamination of milk is started at the farmer level and leads to poor quality milk. Thus, proper awareness programs should be carried out effectively to educate the dairy farmers on clean milk production.

**Index Terms-** Raw milk, Chilling centers, Bacterial count, Coliform count

## 1. INTRODUCTION

Milk is almost complete food for offspring of mammals. It contains a balanced form all the necessary and digestible elements for building and maintaining the human and animal body. In addition, it contains immuno-globulins which protect the newly born against a number of diseases [1]. The dairy industry plays an important role in the current society. Milk is a complex biological fluid by its nature and a good growth medium for many microorganisms [2], [3]. Bacterial contamination of raw milk can originate from different sources like air, milking equipment, feed, soil, faeces and grass [4], [3].

Because of that, it has become an important vehicle for the transmission of milk-borne pathogens to humans. It is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality [2], [3]. As result of microbial activity, the quality of milk deteriorates and if low quality milk is used for processing, poor quality milk products would be resulted. Therefore, good quality as raw material can produce high quality milk products [5].

The dry zone has become one of the highest milk collecting agro ecological zones in Sri Lanka. According to one of the leading milk processors in Sri Lanka, raw milk collected in Anuradhapura district has shown the higher total bacterial count in milk. It is

very important to maintain the microbiological qualities of raw milk during the entire processing flow. Hence, the aim of this study was to evaluate the microbial contamination of raw milk at farmer level, milk chilling centers and milk transportation vessels and also to identify the root cause of contamination and to suggest possible hygienic expedients to minimize the bacterial load of the raw milk.

## 2. METHODOLOGY

This study was carried out at selected chilling centers in Anuradhapura district located in North Central Province, Sri Lanka. Milk samples were collected from 11 chilling centers, 63 farmers and 03 transportation vessels from their front and rear parts. When milk samples were collected, bottles with 60 ml of volume were taken airtight. The sample bottles were placed in a sample case or iced box and transported to the testing laboratory immediately after sampling. The storage temperature after sampling was reached as quickly as possible and maintained between 0 and 4 °C. Samples were examined within 5-6 hours of collection. All the samples were pre heated to room temperature placing in a water bath before testing.

### *Standard Plate Count (SPC)*

The total bacterial count was measured by adding 1 ml of milk sample into sterile test tube having 9 ml of 0.1% peptone solution. It was thoroughly mixed and serially diluted up to  $1:10^{-7}$  [6], [7]. Each analysis was made in triplets using  $1:10^{-5}$  and  $1:10^{-6}$  serial dilution. One ml diluted sample was pour plated using 15-20 ml of Standard Plate Count Agar which was prepared according to the guidelines given by the manufacturers and sterilized by autoclaving at 121°C for 15 minutes. The plated samples were allowed to solidify and then incubated at 30 °C for 48 hours under aerobic condition. Colony counts were made using the colony counter [6], [7].

### *Total Coliform Count (TCC)*

One ml of milk sample was added to sterile test tube having 9 ml of 0.1% peptone solution and thoroughly mixed. It was serially diluted up to  $1:10^{-7}$ . Each analysis was made in triplets using  $1:10^{-5}$  and  $1:10^{-6}$  serial dilution. One ml diluted sample was pour plated using 15-20 ml of MacConkey Agar medium which was prepared according to the guidelines given by the manufacturers and sterilized by autoclaving at 121 °C for 15 minutes. The plated samples were allowed to solidify and then incubated at 30 °C for 24 hours under aerobic condition [6], [7]. Colony counts were made using the colony counter and the number of microorganisms (colony forming units) per ml of milk was calculated using the following formula [8], [7].

$$\text{Count} = \frac{Sk}{(n1 + 0.1n2)} \times d$$

Where,

Sk = sum of all colonies counted (between 10 and 300)

n1 = number of plate from the lowest dilution used for computing the count,

n2 = number of plates in the next dilution factor used for computing the count,

d = reciprocal of the dilution factor of the lowest dilution used for computing the count corresponding to n1.

### *Data Analysis*

Standard plate count and total coliform count data were analyzed using Poisson Regression in GENMOD procedure in SAS. Descriptive statistics of standard plate count and total coliform count data were obtained using Minitab 17.

### 3. RESULTS AND DISCUSSION

**Table 1: Descriptive statistics of Standard Plate Count (SPC) of the tested milk samples**

Group	Acceptable	Mean	St. Dev.	Minimum	Maximum
	standard value	Log			
	Log <sub>10</sub> CFU/ml	Log <sub>10</sub> CFU/ml			
Low risk chilling centers	5.48	6.80	± 0.97	6.073	8.658
High risk chilling centers	5.48	7.39	± 0.78	6.837	8.737
Total chilling centers	5.48	7.07	± 0.89	6.073	8.737
Transportation vessels	5.48	7.08	± 0.37	6.390	7.438
Low risk farmers	5	6.54	± 0.72	5.214	7.826
High risk farmers	5	6.53	± 1.38	0.000	8.308
Total farmers	5	6.53	± 1.19	0.000	8.308

(Sources of standard values: [9]; [10]; [11])

The table 1 shows the descriptive statistics for Standard Plate Count (SPC) of the tested milk samples. This revealed that higher total aerobic bacterial count in all the sample groups ranging from  $6.53 \pm 1.38 \text{ Log}_{10}\text{CFU/ml}$  to  $7.39 \pm 0.78 \text{ Log}_{10}\text{CFU/ml}$  with respect to the acceptable standard value. The maximum number of SPC was found in high risk chilling centers ( $8.737 \text{ Log}_{10}\text{CFU/ml}$ ) while the minimum was recorded in transportation vessels ( $7.438 \text{ Log}_{10}\text{CFU/ml}$ ). It showed the actual risk status. Further, the mean number of SPC increased from farmer level to transportation vessels which reflected the contamination of raw milk occurred at each and every handling point and resulted poor hygienic quality of raw milk.

According to [7], the overall mean total bacterial count of milk was  $7.58 \pm 0.09 \text{ Log}_{10}\text{CFU/ml}$ . The present study showed the lower values compared to above. The average total plate counts of milk samples from farmers, dairy cooperatives and hotels were  $6.88 \pm 0.31 \text{ Log}_{10}\text{CFU/ml}$ ,  $7.10 \pm 0.79 \text{ Log}_{10}\text{CFU/ml}$  and  $7.54 \pm 0.26 \text{ Log}_{10}\text{CFU/ml}$ , respectively as per [12]. According to [13], the overall mean total aerobic bacterial count was  $6.67 \text{ Log}_{10} \text{CFU/ml}$ . [11] also reported that bacterial count ranged from  $7.36 \pm 0.17$  to  $7.88 \pm 0.13 \text{ Log}_{10} \text{CFU/ml}$  of raw cow's milk. However, values reported by the present study were lower than previously reported.

**Table 2: Descriptive statistics of Total Coliform Count (TCC) of the tested milk samples**

Group	Standard	Mean	St. Dev.	Minimum	Maximum
	value Log	Log			
	Log <sub>10</sub> CFU/ml	Log <sub>10</sub> CFU/ml			
Low risk chilling centers	1.69	4.02	± 3.40	0.00	8.65
High risk chilling centers	1.69	5.84	± 3.54	0.00	8.83

Total chilling centers	1.69	4.85	± 3.42	0.00	8.83
Transportation vessels	1.69	6.22	± 0.66	4.959	6.825
Low risk farmers	1.69	6.14	± 0.97	4.134	7.530
High risk farmers	1.69	5.72	± 2.37	0.000	8.428
Total farmers	1.69	5.87	± 1.99	0.000	8.428

(Sources of standard values:[9]; [10]; [11]).

The table 2 shows the descriptive statistics for Total Coliform Count (TCC) of the tested milk samples. The higher total coliform count was observed in chilling centers, transportation vessels and farmers with compared to the standards. The coliform risk was higher in transportation vessels ( $6.22 \pm 0.66 \text{ Log}_{10}\text{CFU/ml}$ ) and low risk farmers ( $6.14 \pm 0.97 \text{ Log}_{10}\text{CFU/ml}$ ).

[7]reported that the overall mean coliform count of milk was  $4.49 \pm 0.11 \text{ Log}_{10}\text{CFU/ml}$ . [12] also reported the average coliform counts were  $5.57 \pm 0.22 \text{ Log}_{10}\text{CFU/ml}$  at farm level,  $5.63 \pm 0.56 \text{ Log}_{10}\text{CFU/ml}$  for dairy cooperative and  $5.37 \pm 0.19 \text{ Log}_{10}\text{CFU/ml}$  for hotels. The values reported by the present study were similar to those of previously reported values. The coliform count ( $5.87 \pm 1.99 \text{ Log}_{10} \text{CFU/ml}$ ) at farmer level in the present study was far lower than the values reported by [13] who reported coliform count ranged from  $7.18 \pm 0.14$  to  $7.46 \pm 0.14 \text{ Log}_{10} \text{CFU/ml}$  of raw cow’s milk.

**Table 3: Comparison of SPC and TCC in different sampling groups**

Groups of Comparison	SPC		TCC	
	Chi-Square	Pr>ChiSq	Chi-Square	Pr>ChiSq
Low and high risk chilling centers	5.134E8	<.0001	1.439E9	<.0001
Low and high risk farmers	7.182E8	<.0001	8.26E8	<.0001
Total farmers and total chilling centers	3.513E7	<.0001	1379010	<.0001
Total chilling centers and total transportation vessels	4.326E7	<.0001	1533796	<.0001

The table 3 shows the comparison of SPC and TCC data in different sampling groups. SPC and TCC were significantly differed ( $\text{Pr}>\text{ChiSq}<.0001$ ) between low and high risk chilling centers, low and high risk farmers, total farmers and total chilling centers, total chilling centers and total transportation vessels. This revealed that microbial contamination was occurring at every stage of milk handling.

#### 4. CONCLUSIONS AND SUGGESTIONS

The quality of milk produced in the study area is poor because of high load of total bacterial count (SPC) and total coliform count (TCC) of the tested milk samples with respect to the international standards. The high load of bacterial count indicates the microbial contamination at every point of milk handling. To minimize the microbial contamination and to produce good quality milk, good health status of animals, hygienic practices during milking, storage and transportation should be maintained. Bulk milk quality checking should be done routinely. Proper standards should be established. Proper awareness programs should be initiated to educate the dairy farmers and milk producers.

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