Quality Survey of Raw Milk Samples in Eastern Nepal: A Microbiological Assessment in Milk Chain System

Regmi S., MSc^a, Shah P. K., MSc^b, Ranjit D. K., MSc^c, & Adhikari R. P., MSc^d

Abstract		
Introduction	A complex system of raw milk collection is being practiced in Nepal. Maintaining an acceptable quality has been a major challenge for Nepalese dairy professionals.	
Objectives	To determine the load of total and coliform bacteria in different points of milk chain system, which were considered as the vulnerable points for contamination.	
Methods	Simple methods for total plate count and coliform count were adopted. Samples, starting from individual farmers to processing plants, were analyzed to determine the possible source of contamination.	
Results	The milk, which was received by MPCs and processing plants, was found to be highly contaminated in comparison to the milk from other points of the milk chain system.	
Conclusion	Farmers and milk carriers (mostly tankers) were seen as important milk contaminating factors. Therefore, the provision of training and regular monitoring and supervision of the facilities is imperative for this group of population.	
Key words	Milk Chain System, Total bacteria, Coliform bacteria Abbreviation key: MPCs = Milk Producers Cooperatives, CC = Chiling Center, PP = Processing Plant	

Introduction

Over the decade, Nepal has been practicing a mechanized process of milk collection and transportation named as 'Milk Chain System'. This kind of the system is being practiced only in limited part of the country so far. Different farmers, Milk Producer's Cooperatives, and the Chilling centers are in action to facilitate the system to flow. Milk Chain System is a complex milk flow mechanism which includes a long successive process from milking, collection, transportation, processing, production and ultimately distribution to consumers.

Fresh, non-pasteurized milk generally contains varying numbers of microorganisms, depending upon the care employed in milking, cleaning and handling of milk utensils. Nearly all of the changes which take place in the taste, odor or appearance of milk after it comes from the cow are the result of the activities of these organisms. Since the organisms are widely distributed in nature, it is conceivable that they may be introduced in to Milk Chain System from a great variety of sources. These sources may be grouped

conveniently from the standpoint of dairy practices as exterior of the body, atmosphere, utensils, various ingredients added to dairy products and the milker or handler's hygiene.

Maintaining milk quality is the major challenge in a dairy industry and it plays vital role for the longevity of milk and its products. To be known what exactly is happening in the milk chain system, it is important to assess different chemical as well as microbiological parameters of the milk in the suspected or potential points. Therefore, to identify the possible point of contamination in the system this research was designed. It focuses on the assessment of bacterial and coliform load in the system starting from farmers to milk processing plant.

Materials and Methods

1. Study areas:

Focal site: Biratnagar milk supply scheme, Biratnagar.

Sampling sites: Milk Producers' Cooperative, Chilling centers, and private collection centers of Ilam, Jhapa, Sunsari,

^a Research Officer, Visceral Leishmaniasis Research Project, Institute of Medicine, Tribhuvan University. GPO Box-5890, Kathmandu, Nepal. E-Mail: sandeshregmi@hotmail.com

b Lecturer, Department of Microbiology, Tri- Chandra College.

^c Lecturer, Department of Microbiology, National College.

^d Lecturer, Central Department of Microbiology.

Morang, Dhankuta, Saptari and Dhanusha districts etc.

2. Milk sampling and practice observation:

A total of 113 raw milk samples were collected in the entire study period starting from 1st June, 1999 to 1st July, 1999. Sterile 50 ml sampling bottle was used for individual sample. To follow the milk chain system, following are the sources of raw milk samples.

- a. Farmer's milk container.
- b. MPCs milk container (dispatch milk sample).
- Storage tanks and chilling vats (dispatch milk sample).
- d. Milk tanker (processing plant receive sample).

In addition to sampling, farmer's practices on cleanliness of utensils, milking, storage, transportation etc are also observed.

3. Storage of Milk samples:

It was not possible to process the samples immediately. So, to keep the quality of milk as received at the collection site, samples were kept under refrigeration (0 to -3°c).

4. Processing of samples:

After having been received in base laboratory (Med-Micro), all the samples were subjected for total bacterial load determination in Total Plate Count Agar and total coliform determination in Violet Red Bile Agar. Methods described in Quality control Handbook, Dairy Development Corporation, Nepal and Manual of Food Quality Control, Microbiological Analysis, FAO, Rome were adopted for the enumeration.

Results

Table 1: Consolidated result

Raw milk sample source	Average bacterial load	Average coliform load
Individual farmer	5.5x10 ⁴ (n=90)	1.6 x10 ⁴ (n=90)
MPCS dispatch	$15.4 \times 10^4 (n=18)$	$7.5 \times 10^4 (n=18)$
MPCS receive at Chilling center	16.0 x10 ⁴ (n=18)	14.4 x10 ⁴ (n=18)
Chilling center (CC) dispatch	$10.7 \times 10^4 (n=4)$	$20.5 \times 10^4 (n=4)$
Processing plant (PP) receive	$32.0 \times 10^4 (n=1)$	$30.0 \times 10^4 (n=1)$

Total bacterial V s total coliform load in Ra



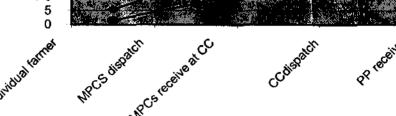


Fig-1

Sampling source

131 raw milk samples were assessed to determine total load of bacteria as well as total coliform. Of the total, 68.7% samples were from individual farmer, 13.7% from MPCs, 13.7% same sample at Chilling center, 3.0% samples from chilling vat and 0.8% from processing plant.

The average bacterial loads were 5.5x10⁴, 15.4 x10⁴, 16.0 x10⁴, 32.0 x10⁴ in raw milk sample from farmer, MPCs, Chilling vat and processing plant respectively. Similarly, the average loads of coliform bacteria in same sources were 1.6 x10⁴, 7.5 x10⁴, 14.4 x10⁴, and 30.0 x10⁴ respectively.

From the result, it was found that raw milk samples were highly contaminated both by general as well as coliform bacteria. The load of bacteria was gradually increased as it passed each and every point.

Discussion

Analysis of raw milk samples in different checkpoints of milk chain system showed that the samples were heavily contaminated by both coliform and general bacterial load. Raw milk from farmers and the primary collectors (before entering in the milk chain system) was found to be contaminated by relatively lower number of bacteria than the number found in the succeeding checkpoints. But when it enters in to the chain system, the microbial number also fluctuates and it usually increases or decreases according to the condition of storage. This can be explicated from the above graph. The bacterial growth pattern is relatively constant in the samples from MPCs dispatch and its receive in the chilling center while the pattern is almost exponential type in the other succeeding points of the system. This implies that the time lag between farmers to MPCs and between chilling center to processing plant is major factors to be responsible for the milk contamination.

After observing the milking practices at noon and evening, the research team seriously felt the urgent need of chilling vat and its lack was the major cause of milk degradation. Moreover, it was also found that the milk had to be left for 10 to 15 hours at night before being transported to the chilling center. In most of the cases, the narrow opening utensils were in practice for milking, handling and transportation. Apparently it was seen that cleaning and washing such utensils was almost impossible because of inaccessibility. In majority of cases, part of spoiled milk that deposited in the milking utensils might have contributed greatly in milk contamination.

Unsatisfactory hygiene and sanitary conditions of the raw milk suggest the existence of great risk to the health of the consumers, especially when the product is taken without being boiled. The research further intensify that interior and exterior of the body, atmosphere, utensil, various ingredients added to dairy products and milkers or handlers are the major sources of milk contamination.

Therefore, the wiping of the udder and flanks with a clean, damp cloth and avoiding narrow-mouthed pail are extremely important precaution that must be taken if one is to produce a clean, low count milk².

Spoilage organisms may come from the excreta of the cow and be present in large numbers when hygienic conditions in the dairy are neglected and especially when temperatures are high enough to encourage the growth of these intestinal types. Hygiene and sanitation are the solutions for reducing or eliminating this source microorganisms³. The filth from the body of the cow must be reduced as much as possible. During raw milk sampling, members of the research team visited different person, household, animal shed, locality, etc. Thus the research findings were based on the practical experiences and observed existing practices. The findings showed that an unhygienic animal shed, an unhygienic container, the way of milking and the handling of milk are the sources of the milk contamination. Therefore, it is suggested that in order to reduce the milk contamination, first of all the farmer (owner of the milking animal) should have the basic knowledge of contamination, the fate of contaminated milk and the possible result of the use of NaHCO₃ as acid neutralizing agent. To get rid from this problem, the individuals who are actively involve in the chain mechanism of the milk flow should strictly follow the rules and regulation that has been formulated. In the second step, persons who are involved in this milk flow chain should be aware of the possible contamination from the different sources. The success in this regard can be achieved by making the respective people trained in their respective field. The volume of milk and its contamination becomes higher and higher as it passes in the chain. The human activities, the mechanical activities and the influence of the environmental activities also increase the chances of contamination. When the milk comes to chill at around 0°C, the possibility of the microbial growth at this temperature is greatly reduced and of course further degradation may be interrupted. But the field observation showed that milkchilling facilities in Nepal are very limited and only confined to the limited areas.

Being highly nutritious growth medium for most of the bacterial organisms, the milk always possess the high risk of the contamination. It has been seen that heavy contamination by the bacterial organisms may alter the chemical properties of the milk and ultimately the whole milk has to be disposed. This type of milk degradation can be seen very commonly in our community, which is unfortunate from the economic point of view. Hence, the existing practices of milking, its handling and processing, the time required to transport and the temperature during transportation are the major factors for consideration by Nepalese dairy industries.

100% samples under this study were found out of quality. Both tests (the total bacterial count and the coliform count) showed that the raw milk was contaminated with very high number of the bacterial organisms. Because of lack of previous data or research in this discipline we couldn't be able to compare the present result. However, the research findings can be studied with the following references of other similar studies conducted in different parts of the world.

A research group found total bacterial counts (TBC)< or = 30,000 cfu/ml in 63% of the raw milk samples. Coliform group of organisms was present in all samples, but 65-71% of samples had <100 coliforms/ml⁵. Similar finding was seen in another research article which showed that Coliform group of organism was present in most of the raw milk test samples, but 84% of the samples had counts<100cfu/ml. About 80% of the samples had $< or = 10 E.coli cfu/ml^6$. Another raw milk survey study showed that of the 129 raw milk samples, 25%, 37.2%, 5.4%, 7.7%, 18.6%, 1.6% samples were positive for E.coli, Salmonella spp., Shigella spp., Klebsiella spp., Citrobacter spp. and Pseudomonas spp respectively?

A similar study was conducted in Ethiopia and found that the lowest count registered for raw milk samples was 4×10^7 cfu/ml. 360 raw milk samples were collected from three milk centers and analyzed for the presence or absence of coliform bacteria. It was found that the number of coliform organisms/ml of raw milk sample varied from 10^5 to 10^7 depending on the season.

The effect of the teat washing and drying on the bacterial numbers in bulk milk was compared with that of no teat preparation in eight commercial herds over a one-year period. Teat washing and drying of cows housed during winter reduced the total counts by 40% and Streptococcal and Coliform counts by 50%. Bacteriological counts were significantly lower in cows at pasture during the summer and there was no reduction in count due to the teat washing and drying. Bacteriological

Reference

- Badini K. B., A. F. Nader, L. A. do Amaral, P. M. Germano. Health risk due to the consumption of raw milk commercialized without due authorization: Rev Saude Publica. 30: 549-52.
- MacKinnon C. H., G. J. Rowlands, and A. J. Bramley. The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds: *J Dairy Res.* 1990; 57: 307-18.

counts increased at each point as the milk passed through the milking mechine.

All the references mentioned above indicated that milk has been spoilt by organisms even in the developed countries. It was also found that, in some cases, the density of total bacteria or coliform was higher than that found in our study. Milking and other handling equipment are the potential source for increasing the bacterial load in the developing countries like Nepal where the traditional manual method of milking is still in practice. Addition of contaminated water, transportation time, cleanliness of utensils and the temperature of the milk are the important sources of bacterial contamination found in this research. Therefore, if we have more chilling vats, improved milking practices and clean milk containers, it is likely that the magnitude of milk contamination and degradation can largely be reduced.

Conclusion

In the milk chain system, the bacterial load from MPCs to chilling center was seen relatively constant. But other two different points were seen to be reformed urgently. Milk Producers Cooperatives and its entire milking operation was questionable, similarly the chilling center's operation and tanker parties were also equally responsible for the milk deterioration. Especially when the government or related authority tries to implement the quality based program, these two points need to be considered as the target in the milk chain system.

Acknowledgement

Authors would like to acknowledge NDDB/DANIDA/DSP for financial support and Med-Micro/Bio service Laboratory for laboratory accommodation. We would also like to thank all the staff members of MPCs, Chilling centers and processing plant in Biratnagar for their cooperation during the research.

- de Centorbi O. P., A. C. de Guzman, A. A. de Cuadrado, A. L. Laciar, L. E. Alcaraz, M. C. de Milan, and N. B. de Pederiva. Determination of the sanitary quality and detection of Salmonella spp and Yersinia enterocolitica in ice cream: Rev Argent Microbiol. 1989; 21: 63-9.
- Headrick M. L., S. Korangy, N. H. Bean, F. J. Angulo, S. F. Altekruse, M. E. Potter, and K. C. Klontz. The epidemiology of raw milk-associated foodborne disease outbreaks

- reported in the United States, 1993 through 1992: Am J Public Health. 1998; 88: 1219-21.
- Rea M. C., T. M. Cogan, and S. Tobin. Incidence of pathogenic bacteria in raw milk in Ireland: *J Appli Bacteriol* 1992; 73: 331-6.
- Desmasures N., F. Bazin, and M. Gueguen. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy: J Appl Microbiol. 1997; 83: 53-8.
- Regmi, S., P. K. Shah, D. K. Ranjit, R. Lamichhane, S. Ranjit, and R. P. Adhikari. Prevalence of different pathogenic enteric bacteria in raw milk samples from different

- geographical regions of Nepal: Journal of Nepal Public Health Association. 2001;1:44-46.
- 8. Mackinnon C. H., G. J. Rowinade, and A. J. Bramley. 1990. The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds: J Dairy Res. 57: 307-18.
- Regmi, S., P. K. Shah, D. K. Ranjit, R. Larnichhane, S. Ranjit, and R. P. Adhikari. 2001. Prevalence of different pathogenic enteric bacteria in raw milk samples from different geographical regions of Nepal. Journal of Nepal Public Health Association. 1:44-46.

FOR DISPLAY ONLY.