

Hygienic Quality of Raw Cows' Milk at Farm level in Dharan, Nepal

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The study was aimed to assess the quality of raw milk and hygienic status of dairy farms at household level in Dharan due to the fact that milk produced in Nepal by the informal sector is not regulated by any agency and such milk may pose a health hazard due to contamination with pathogens. This study was conducted between February and March, 2019 and accomplished in three phases viz. field survey, physicochemical analysis with clot-on-boiling (COB), alcohol and acidity tests as well as microbial examinations with aerobic mesophilic bacterial count (AMBC) and coliform count (CC). The survey findings revealed that majority of the dairy farms still practiced traditional methods as a result of which earthen floor with no bedding materials were seen on majority of the barns. Although 80% farmers cleaned their barn on daily basis but 54% calves were not in a good hygienic condition. Also, 90% farmers allow their calf to suck the teats; 60% supplemented their cows with locally available feed and 87% had municipal tap water facility. The overall mean of AMBC and CC were 7.5×10^4 and 4.5×10^4 cfu/ml respectively ($p > 0.05$) which were much higher than standard set by Institute of Medicine and National Research Council (US). The overall mean of % lactic acid was 0.20% and predicted probabilities of COB and alcohol positive tests were 13% and 33% respectively. The results obtained from the study indicated that the current situation is critical and needs hygienic milking and handling practices.

Keywords: Acidity test, Aerobic mesophilic bacterial count, Alcohol test, Clot on boiling test, Coliform count

Introduction

Milk is most likely an extremely perishable food and improper handling may cause an outbreak to public health problems and economic losses, thus requiring hygienic vigilance throughout the entire milk chain starting from producer to consumer (Hayes and Boor, 2001). Contamination of milk may occur through various sources. May be through infected cow with tuberculosis, brucellosis, and mastitis and also from milk handlers infected with typhoid fever, diphtheria, dysentery, and scarlet fever (Jay *et al.*, 2005). It is common that dairy cattle and their farm's surroundings may contain many pathogens such as *Listeria*, *Salmonella*, and pathogenic *Escherichia coli*. Raw or inadequately pasteurized milk may contain toxin producing *E. coli*, *Salmonella*, *Listeria monocytogenes* and others (Pal *et al.*, 2016).

In a survey of raw milk in Nepal, it was found that out of the 129 samples, 25%, 37.2%, 5.4%, 7.7%, 18.6%, 1.6%, were positive for *E. coli*, *Salmonella spp.*, *Shigella spp.*, *Klebsiella sp.*, *Citrobacter spp.*, *Pseudomonas spp.* respectively (Regmi *et al.*, 2001). Air, feed, grass, soil, milking equipment and faeces may be the primary sources, through whose contact other sources may lead to contaminate raw milk with different microorganisms (Swai and Schoonman, 2011).

Poor pre-milking udder hygiene that fails adequately to clean dirty udders may also result in the introduction of vegetation, soil, and bedding material and their associated microorganisms into the milk (Hayes and Boor, 2001). Such foreign matters and contaminations in the milk may lead to concerns regarding consumer health (Lemma *et al.*, 2018). Hence the safety of milk and its products is of great concern around the world. This is even bigger concern in developing and under developed nation where milk and its products are

prepared in unsanitary conditions (Tassew and Seifu, 2011). According to Ministry of Finance (MOF, 2019), Nepal's total production of milk in fiscal year 2018/19 was 1,475,333 metric tons (MT), with cow milk 590,133 MT (40%) and buffalo milk 885,200 MT (60%).

FAO (2010) found that out of the total milk production in Nepal, only about 10% of milk is estimated to be used by the recognized dairy sectors and rest (90%) goes to informal sector, milk hawkers and small dairy cooperatives. This is a potential threat to public health in massive scale due to growth of bacterial pathogens in milk. It was found that the milk supplied by various dairies in Kathmandu valley is not free from microorganisms (Arjyal *et al.*, 2004) which are even a bigger concern for consumers' health in Nepal.

This study was performed to observe overall hygienic practices and magnitude of bacterial contaminants in raw cows' milk produced and supplied along the informal dairy value chain (producers to consumers) in Dharan. This study reports on an evaluation of the microbial quality of raw milk supplied to the market and hygienic practices at farm level in Dharan.

Materials and methods

The area of the study was Dharan sub-metropolitan city located in Sunsari district, Koshi zone, Province no. 1, Nepal. The specific study sites were ward no. 1, 3, 6, 13, 15 and 16 of Dharan, these wards are selected by random sampling out of 20 wards.

Materials

Ethanol was purchased from Changshu Hongsheng Fine Chemical Col Ltd, China, sodium hydroxide was from

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Qualigens, India and Violet Red Bile Agar (VRBA) and Plate Count Agar were from Himedia. The Colony Counter was manufactured by Synbiosis USA.

Methods

Study design

Farmers involved in this study were individual farmers having three or more than three lactating cows in a farm, it was assumed that 5-6 liters milk was consumed within household level and do not go to the milk chain. Questionnaires were distributed and raw milk samples were collected in morning at farm from the common pool container prior to milk delivery to the market. The study was conducted on lactating cross breed cows (Holstein and indigenous cross and Jersey and indigenous cross) and indigenous breed. Preliminary survey showed that there were almost 116 small farms, therefore sample size was made to 90.

Data collection and milk sampling

A. Questionnaire based survey

The structured questionnaire (Appendix I) were prepared as mentioned on 'Standardized Protocol to Develop Dairy Farm Management Questionnaires for Observational Studies' (Scholl *et al.*, 1992) and it was similar as mentioned by Res *et al.*(2018). The questionnaire was asked through face-to-face interview. During questionnaires survey, direct observation on general cleanliness, hygienic conditions of barn and milk handling practices were done and noted. The questionnaire was used for collecting information on possible factors causing risks for microbial contaminations in milk. The risk factors considered in this study were sanitary conditions of the barn and milking surroundings, milking cows' udder and hygiene of milk handlers. It was also considered milking equipment hygiene with emphasis to milking procedures hygiene and hygiene of milk handling practices, conditions of utensils used for milking and milk storage as well as uses of milk for selling or domestic purposes. Furthermore, information about milk consumption behaviors and awareness regarding the consumption of raw milk associated with the risk of zoonotic diseases was also taken into consideration.

B. Milk sampling

Raw milk samples were taken from ward no. 1, 3, 6, 13, 15, and 16. Five households were selected from each ward. Therefore, total of 30 milk samples were collected at once in morning at a time, which was repeated for 3 times for one-month data (one sample set in 10 days) i.e. total of 90 samples. Pooled milk was collected as sample within 2 hours after milking, from the farmer's delivery containers. The samples were collected in sterilized universal bottles (Purwanchal Scientific Pvt. Ltd., Biratnagar) aseptically, placed in an icebox and carried to the laboratory and stored in refrigerator at 5°C. Analysis was done one hour after sampling in duplicates. All the analyses were done within 8 h of sampling.

C. Physicochemical and microbial analysis

The tests considered were Aerobic Mesophilic Bacterial Count (AMBC), Coliform Count (CC), acidity test, alcohol test and clot-on-boiling test. Each analysis was made in duplicate, with precession of more than 95%.

a) Determination of aerobic mesophilic bacterial count (AMBC)

The determination of AMBC was done by the method suggested by Mhone *et al.* (2011).

Table 1

Sampling plan (one set of samples in every 10 days for one month)

SN	Ward no.	Cow Breed type	Number of samples
1	1(5 house hold)	2 local/3 cross breed	5
2	3(5 house hold)	2 local/3 cross breed	5
3	6(5 house hold)	2 local/3 cross breed	5
4	13(5 house hold)	3 local/2 cross breed	5
5	15(5 house hold)	3 local/2 cross breed	5
6	16(5 house hold)	3 local/2 cross breed	5

b) Determination of coliform plate count

The procedure given by NDDB (2001) was followed for the determination of coliform plate counts.

c) Determination of % Lactic acid

Lactic acid was determined according to method mentioned by Chaudhry *et al.* (2015).

d) Alcohol test

Alcohol test was done according to method mentioned by Chaudhry *et al.* (2015).

e) Clot on Boiling test (COB)

COB test was done according to method mentioned by Chaudhry *et al.* (2015).

f) Data handling and analysis

Analysis of variance for aerobic mesophilic bacteria count, coliform count and % lactic acid was carried out; predicted probabilities for clot-on-boiling and alcohol were estimated using IBM SPSS version 20 (IBM, 2015). The number of microorganisms (colony forming units) per mL of milk was calculated using the following formula (NDDB, 2001).

$$\text{Count} = \frac{\sum c}{(n_1 + 0.1 \times n_2) \times d}$$

where, $\sum c$ = sum of all colonies counted (between 10 and 150); n_1 = number of plates from the lowest dilution used for computing the count ; n_2 = number of plates in the next dilution factor used for computing the count; d = reciprocal of the dilution factor of the lower dilution used for computing the count corresponding to n_1 .

Results and Discussion

Results of survey

A. Dairy cattle housing and cleaning practices

According to the current study, 16% of farmers constructed their farm with concrete materials which facilitates easy

cleaning, while the floor of about 84% of cow shed had earthen and covered with manure since they do not remove the manure completely during cleaning and found in poor hygienic state. It was observed that 30% of the farmers used cereal straw and grass as bedding material for their animals and had good conditioned barn (Table 2). Also, 70% households did not use any bedding material at all and milking cows lies on muddy floor. Teats and udders of cows were soiled while lying in such stalls. About 20% of the respondents clean the barn thrice a week while 80% reported that they clean daily. Cleaning of the barn with water was done on average every two weeks. Such dirty environments are also likely to be sources of milk contaminations. Similar observations have been reported in Tanga (Shija, 2013). The study also showed that 54% of the farmers did not keep calves in a good hygiene, while about 46% did very well. Further, 90% of the farmers allow their calf to suck the cow's teats.

In general, providing proper shelter for animals has not been given the required attention. Housing conditions in many of households were dirty and unclean. This may have a negative impact on the quality of milk and milk products produced and processed. Proper and clean housing environment is a prerequisite to produce milk and milk products of acceptable quality (Asaminew, 2007).

B. Feeding and watering practices

Almost all respondents reported that they allowed their cattle freely graze the natural grazing lands (Table 3). However, about 60% of the interviewed producers provide their cows with locally available feed resources. There were different sources of water used for cattle i.e., 87% had access to municipal tap water, 10% used river water while 3% of them used spring water.

There is currently a consumer perception that milk from cows on pasture is more natural than that from more conventional indoor ration feeding systems (Verkerk, 2003). Feed system

has a significant effect on milk yield and milk solids yield (O'Callaghan *et al.*, 2018).

C. Milking and Hygienic Practices

Milking was done manually mostly by women. Cows were usually milked twice a day by 90% farmers while 10% milked three times a day (Table 4A). Plastic buckets and metal containers were used during milking; 87% use plastic bucket while 13% used metal container. About 90% farmers practiced washing of their milk utensils daily, while 10% cleaned three times a week before milking; the cleaning was not effective and utensils were not properly dried. It was observed that milkers' dip their fingers in the milking vessel to moisten teats of the cows with the intention of facilitating milking. All milkers' used plastic type of container to transport milk. Similar observation was also reported by Shija (2013), who observed high microbial load in milk which was correlated with narrow necked plastic containers used in handling of milk.

Among the respondents across the 6 wards, 70% washed their hands before milking while 30% did not wash. However, none of the interviewees washed their hands between milking of different cows. About 10% did not wash udder before milking while the 90% did wash. Similarly, 90% of all the interviewees did not use towel to dry udder after washing rather they massage the udder with bare hands while, about 10% reported that they use local material, *paral* (hay) for teat and hand drying purposes.

Generally, it was observed that the milking environments and utensils were unhygienic indicating the possibilities for microbial contaminations of milk. In addition, milk cooling was not done after milking and before delivery due to lack of chilling facilities.

Table 2

Types of housing, cleaning practice and calf management in 6 wards of Dharan.

Variables		Ward no.						Total (%)
		1 (n=15)	3 (n=15)	6 (n=15)	13 (n=15)	15 (n=15)	16 (n=15)	
Type of Barn	Concrete floor	3	3	2	2	2	2	16
	Earthen floor	12	12	13	13	13	13	84
Condition of Barn	Grass bedding	4	5	3	6	5	4	30
	Muddy bedding	11	10	12	9	10	11	70
Frequency of Barn cleaning	Daily	10	11	12	13	14	12	80
	3 times a week	5	4	3	2	1	3	20
Calf Cleanliness	Clean body	5	6	7	8	9	6	46
	Soiled body	10	9	8	7	6	9	54

n = number of respondents in a ward.

It was reported by Depiazzi and Bell (2002), that pre-milking udder preparation and teat sanitation play important part in the microbial load of milk, infection with mastitis, and environmental contamination of raw milk during milking. Cleaning the udder of cows before milking is important since it could have direct contact with the ground, urine, dung and feed refusals while resting.

Lack of washing udder before milking can impart possible contaminants into the milk. The current study is in agreement with other reports (Derese, 2008). Production of milk of good hygienic quality for consumers requires good hygienic practices (clean milking utensils, washing milkers' hands, washing the udder and use of individual towels) during milking and handling, before delivery to consumers or processors (Getachew, 2003).

D. Public health aspect

As given in Table 4B, about 7% of the interviewed producers consumed raw milk while the remaining 93% boiled raw milk before consumption. Although about 83% of the respondents were aware about the risk of public health hazards associated with consumption of raw milk, some of them did not boil milk for consumption and 50% of the respondents reported they were suffered from food borne infections as symptoms included nausea, vomiting, stomach cramps, and diarrhea. All dairy cow owners milk their cows by hand and did not cool the milk after milking. It was common that fresh milk was mixed with milk left over from previous batch, and milk of different cows of the same farm was mixed together.

The consumption of raw milk with no treatment may pose a public health hazard as a result of poor safety and quality. This habit therefore poses a lot of dangers to consumers in relation to milk-borne diseases (Lues *et al.*, 2003). Elsewhere despite of livestock keepers being aware of the risk of contracting zoonotic infections and milk-borne diseases, the general public still consume raw milk (Mosalagae *et al.*, 2011).

Hence, the present findings showed that there were several practices undertaken at farm level such as type of animal house floor, not washing hands and udder/teats before milking, water used for cleanliness (hands and equipments), type of storage containers used and milk storage duration under ambient temperature that predispose raw milk to microbial contaminations. Apart from that, it was observed that there are traditional ways practiced by individual dairy farmers including the raw milk and milk products consumption.

2. Results of physicochemical and microbiological

A. Aerobic mesophilic bacterial count (AMBC) and Coliform count (CC)

The mean and standard deviation of AMBC by breed wise, and AMBC by ward no. and overall are given in the Table 5. Results of analysis of variance indicated that there was no significant difference in the sample means between cow breed and wards in terms of AMBC as well as CC ($p > 0.05$). The mean value of AMBC and CC of raw milk collected from delivery milk containers were 1.50×10^5 and 8.94×10^4 cfu/mL respectively. The overall values of AMBC observed in the current study were much higher when compared with the tolerable maximum limit given by the Institute of Medicine and National Research Council, US (2003) which are 1×10^5 cfu/mL for producer and 3×10^5 cfu/mL for dairy plant. Similar data were observed by Acharya *et al.* (2017) in Kathmandu valley, Bhattarai and Singha (2010) in Makwanpur district and Koirala (2016) in Pokhara. Generally, the high counts for both Aerobic mesophiles and coliforms in milk is an indication of contamination of milk (Rizani *et al.*, 2018). It is an index of hygienic standard used in the milk production. The sources may include poor farm hygiene, use of improperly washed milking equipment, unsanitary milking practices as well as contaminated water (Lamsal, 2018). A high bacterial count suggests that the milk has been contaminated by bacteria from different possible sources.

Table 3

Feeding and watering practices of dairy cattle in 6 wards of Dharan.

Variables		Ward no.						Total (%)
		1 (n=15)	3 (n=15)	6 (n=15)	13 (n=15)	15 (n=15)	16 (n=15)	
Feeding regime	Grazing natural pasture	15	15	15	15	15	15	100
	Supplemented with local feed	7 (out of 15)	8 (out of 15)	9 (out of 15)	10 (out of 15)	11 (out of 15)	9 (out of 15)	60
Source of water for farm activity	Pipe	15	15	12	12	10	14	87
	River	0	0	3	3	2	1	10
	Spring	0	0	0	0	3	0	3

This may be due the contribution of insufficient pre-milking udder preparation, use of poor-quality water for cleaning without heat treatment and the storage container and time (Jay *et al.*, 2005). As reported by Karns *et al.*(2004), raw milk consumption poses some level of risk but this is not the only reason for concern. In recent years, the safe food supply has become a focal point for public concern. Consumers are more aware of the potential for food-borne pathogens and are concerned that their food is ‘clean and wholesome’ (Shah *et*

al., 2016). Public perception of food quality is critical in the marketing of any product. Therefore, even though pasteurization is an effective control method for bacterial pathogens, it is important to maintain high preprocessing standards and eliminate post pasteurization contamination (Rizani *et al.*, 2018). Additionally, raw milk contaminated with zoonotic pathogens might provide a reservoir for recontamination at milk processing plants (Jay *et al.*, 2005).

Table 4A
Milking and Hygienic practices followed by producers in Dharan.

Variables		Ward no.						Total (%)
		1 (n=15)	3 (n=15)	6 (n=15)	13 (n=15)	15 (n=15)	16 (n=15)	
Milking frequency	2 times	14	14	14	13	13	13	90
	3 times	1	1	1	2	2	2	10
Milking utensils used for milking	Plastic	12	11	13	15	14	13	87
	Metal	3	4	2	0	1	2	13
Cleaning frequency of utensils	Daily	12	13	14	15	15	12	90
	3 times a week	3	2	1	0	0	3	10
Washing hands before milking	Yes	9	10	11	12	9	12	70
	No	6	5	4	3	6	3	30
Washing udder and teats before milking	Yes	12	13	14	13	14	15	90
	No	3	2	1	2	1	0	10
Drying udder and teats before milking	Yes	3	2	1	1	0	2	10
	No	12	13	14	14	15	13	90
Utensils used for transport	Plastics	15	15	15	15	15	15	100
	Metal	0	0	0	0	0	0	0

Table 4B
Public health aspects associated with consumption of raw milk.

Variables		Ward no.						Total (%)
		1 (n=15)	3 (n=15)	6 (n=15)	13 (n=15)	15 (n=15)	16 (n=15)	
Habit of Milk consumption	Raw	1	1	1	1	1	1	7
	Boiled	14	14	14	14	14	14	93
Risk knowledge of raw milk consumption	Yes	10	11	12	13	14	15	83
	No	5	4	3	2	1	0	17
Suffer with food born infection	Yes	6	7	8	9	7	8	50
	No	9	8	7	6	8	7	50

B. Titratable acidity

The mean and standard deviation of lactic acid % by breed wise, by ward no. and overall is given in the Table 6. Results of analysis of variance indicated that there was no significance difference in the samples means between cow breed and ward no. in terms of lactic acid % ($P>0.05$).

Table 5

Mean and standard deviation of AMBC and CC, breed and ward wise in cfu/mL (Mean±SD).

Particulars	AMBC (Mean ±SD)	CC (Mean ±SD)	Sample size
Block Overall	1.50×10^5 $\pm 0.03 \times 10^5$	8.94×10^4 $\pm 0.34 \times 10^4$	N = 90
Breed 1 (Local)	1.49×10^5 $\pm 0.03 \times 10^5$	8.94×10^4 $\pm 0.34 \times 10^4$	n ₁ = 45
Breed 2 (Cross)	1.50×10^5 $\pm 0.03 \times 10^5$	8.92×10^4 $\pm 0.32 \times 10^4$	n ₂ = 45
Breed Overall	1.50×10^5 $\pm 0.03 \times 10^5$	8.94×10^4 $\pm 0.34 \times 10^4$	N = 90
Ward no.1	1.50×10^5 $\pm 0.03 \times 10^5$	8.86×10^4 $\pm 0.34 \times 10^4$	n = 15
Ward no.3	1.50×10^5 $\pm 0.03 \times 10^5$	8.92×10^4 $\pm 0.38 \times 10^4$	n = 15
Ward no.6	1.50×10^5 $\pm 0.02 \times 10^5$	8.78×10^4 $\pm 0.28 \times 10^4$	n = 15
Ward no.13	1.49×10^5 $\pm 0.03 \times 10^5$	8.94×10^4 $\pm 0.26 \times 10^4$	n = 15
Ward no.15	1.50×10^5 $\pm 0.03 \times 10^5$	9.06×10^4 $\pm 0.32 \times 10^4$	n = 15
Ward no.16	1.49×10^5 $\pm 0.03 \times 10^5$	9.04×10^4 $\pm 0.36 \times 10^4$	n = 15

The overall mean titratable acidity of cows' milk produced in the study area was 0.20% which was higher than the value obtained by Mahmood and Usman (2010).

Table 6

Mean and standard deviation of Lactic acid % (Mean±SD), breed and ward wise.

Particulars	Lactic acid % (Mean±SD)	Sample size
Block Overall	0.20±0.04	N = 90
Breed 1 (Local)	0.20±0.04	n ₁ = 45
Breed 2 (Cross)	0.21±0.05	n ₂ = 45
Breed Overall	0.20±0.04	N = 90
Ward no.1	0.20±0.04	n = 15
Ward no.3	0.21±0.05	n = 15
Ward no.6	0.20±0.04	n = 15
Ward no.13	0.20±0.04	n = 15
Ward no.15	0.21±0.05	n = 15
Ward no.16	0.20±0.04	n = 15
Ward Overall	0.20±0.04	N = 90

The high percent lactic acid of milk observed implies to the poor milk handling reflecting the substandard hygienic conditions during production and handling of milk in the study area (Jay *et al.*, 2005). Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid (Gemechu *et al.*, 2015).

C. Alcohol and COB tests

The predicted probability of COB positive test for cow milk samples collected in the study area was 13% and alcohol positive test was 33% (Table 7). Similar findings were reported by Nurliyani *et al.* (2015). The alcohol test is more sensitive than COB test. COB only detects milk which is highly acidic (pH < 5.3). The alcohol test detects even medium acidity milk (pH < 6.4). Therefore, milk which passes the COB test, may fail the alcohol test (Tessem and Tibbo, 2009).

Table 7

Alcohol and COB +ve test.

Particulars	Alcohol +ve %	COB +ve %	Sample size
Block Overall	33	13	N = 90
Breed 1 (Local)	31	9	n ₁ = 45
Breed 2 (Cross)	36	18	n ₂ = 45
Breed Overall	33	13	N = 90
Ward no.1	40	13	n = 15
Ward no.3	40	20	n = 15
Ward no.6	20	7	n = 15
Ward no.13	33	13	n = 15
Ward no.15	33	20	n = 15
Ward no.16	33	7	n = 15
Ward Overall	33	13	N = 90

Conclusions

Based on findings, the present study could be concluded that hygienic and milking practices in Dharan were poor resulting in the production of poor-quality milk. The quality of collected milk samples taking account on the AMBC and CC results were below standards as given by Institute of Medicine and National Research Council (US). Also, higher acidity and positive alcohol test indicate the poor hygienic practice. This may be due to lack of compliance of strict hygienic practices during milk production and subsequent handling. So, it is an indicative of potential public health risk to consumer, particularly if the milk is consumed without boiling or pasteurization.

Recommendation

Our results suggested that the urgent need to implement the awareness campaigns for milk producers about hygienic milking practices and apply effective checkup by food quality controlling authorities throughout the chain to improve microbial as well as keeping quality of raw milk.

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