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Research Article

Microbial quality and handling practices of raw cow milk in North Shewa Zone, Oromia, Ethiopia

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Abstract

Background: Milk is universally accepted as a complete diet that plays a key role to ensure food security. However, the quality of raw cow milk is affected at various stages due to inadequate dairy infrastructure and limited knowledge of hygienic handling practices. Thus, the study aimed to evaluate the microbial quality of raw cow milk and handling practices in the North Shewa zone, Oromia, Ethiopia.

Methodology: Purposive sampling was used to select study districts and participants based on the available dairy production potential, milk market, and collection center. Four hundred participants were selected; 50 raw cow milk samples were collected from households and collection spots in study areas.

Results: Microbial analysis indicated that the mean values of total cell counts were higher in households (4.836±0.206 in log10 CFU/ml) than 4.391±0.15 at collection spots. Similarly, the mean values of *Staphylococcus aurous* were 2.470±0.038 at the household level and 2.249±0.093 at collection spots. The mean values of *E.coli*, yeast count and mould count were however, higher at collection spots (5.414±0.100, 3.372±0.091, 2.670±0.037) than household level (3.343±0.122, 2.944±0.074, 2.479±0.039), respectively.

Conclusions: The higher total counts and mean values of microbial loads of economic and public health significance; like *Staphylococcus aurous* and *E. coli* at household level and collection spots indicated poor hygienic raw cow milk handling practices. Therefore, awareness should be created among the dairy cattle producers and milk collectors to minimize economic losses through milk spoilage with soil microbes and the risk of consumer safety due to zoonotic pathogens.

Abbreviations

APC: Aerobic Plate Count EcC: E.coli Count; GDP: Gross Domestic Product LB: Lactose Broth; MC: Mold Count; MSA: Mannitol Salt Agar PCA: Plate Count Agar; SaC: Staphylococci aureus Count SPC: Standard Plate Count; TAMBC: Total Aerobic Mesophilic Bacterial Count TCC: Total Coliform Count; VRBA: Violet Red Bile Agar YC: Yeast Count

Background

Ethiopia is endowed with the largest livestock population in Africa, comprising approximately 59.5 million heads of cattle, 30.7 million sheep, and 30.2 million goats. Livestock provides 16% of the total GDP (equivalent to 30% of agricultural GDP) and generates 14% of the country's foreign exchange [1]. Dairy production, among the livestock production sector, is a critical issue in Ethiopia; because it is among the main sources of food

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and income. However, the livestock sector has not been fully exploited and promoted in the country [2,3]. Cow milk and milk products generate income for the farm households regularly and provide highly nutritious food for people of all ages, including infants and lactating mothers; reducing the problem of malnutrition among the rural households [4].

Despite the high livestock population, the productivity of the dairy production and benefits obtained from the sector are still very low. For instance, the annual per capita consumption of milk in Ethiopia is very low, (19 liters/person/year) as compared to other African countries like Kenya (120 liters/person/year) and Sudan (180 liters/person/year) [5].

Milk value chains have several outlets through which milk products flow from the producer to the consumer; which affects the quality of milk and transaction costs as well as the potential risk of contamination with pathogens. Subsequently, the safety of dairy products is a major concern around the world, particularly in developing countries where poor handling practices of dairy products from the consumer health point of view may lead to the transmission of various foodborne diseases [6,7].

Recently, an enormous number of smallholder dairy farms have been operating in the North Shewa zone, Oromia, Ethiopia. However, productivity is hindered by several challenges such as shortage of feed, the burden of disease, inadequate veterinary service, and lack of infrastructure coupled with limited knowledge on the hygienic handling practices of raw milk; which could result in public health risks and economic losses [8]. In addition, there is no well-documented information about the microbial quality and handling practices of raw cow milk in the area [9]. Thus, the study was designed to elucidate fundamental information on the microbial quality and assess handling practices of raw cow milk in the North Shewa zone, Oromia, Ethiopia.

Materials and methods

Study areas and design

A cross-sectional study design was conducted in the Kuyu, Girar Jarso, and Wuchale districts of the North Shewa zone, Oromia, Ethiopia. The zone is located 112 km north of Addis Ababa, the capital city of Ethiopia, on the asphalt road connecting Addis Ababa to Gojam. It is located between 9°05' and 10°23'N latitude and 37°57' and 39°28'E longitude. The zone has 13 rural districts, one administrative town (Fitche), 18 towns, and 267 rural and 24 urban kebeles. Its altitude ranges from about less than 1000m to over 3540m. Its annual rainfall is from 600 - 2000mm. The average minimum temperature is 10°C and the maximum temperature is 32°C [10]. The major livestock species managed in the areas include cattle, small ruminants, and equines. The subsector contributes to the subsistence requirement of the population in terms of milk, milk products, and meat [8].

Livestock production is the dominant activity in the zone next to crop production; which accounts 1,676,748 cattle, 118,0430 sheep, 32,4274 goats, 106,472 horses, 8,035 mules, 270881 donkeys, 102,367 poultry and 142,210 beehives (147,268 cultural, 9,101 intermediate and 3,286 modern) in the zone. It is called the milk belt area where 206,000,000 liters of milk per year is produced and more than 60% is supplied to Addis Ababa; because there are a remarkable number of dairy cattle population in the zone; comprising 352,216 local and 1,324,532 crossbreeds. There are 1470 smallholder dairy cattle producers and 30 collectors in the zone [8]. Figure 1 below depicts the map of the north Shewa zone and study districts.

Study participants

The study participants were smallholder dairy cattle producers and collectors in the three selected districts (Kuyu, Girar Jarso, and Wuchale), North Shewa Zone, and Oromia, Ethiopia.

Sampling and sample size determination

A cross-sectional study design was conducted from March 2018 to December 2019. The purposive sampling technique was used to select study districts and study participants based on the potential of dairy cattle production and accessibility to transportation. Three peasant associations were randomly selected from each district. The sample size was determined according to Bartlett, et al. [11]. A list of 1500 smallholder dairy cattle producers and collectors (1470 market-oriented smallholder dairy cattle producers and 30 collectors) was considered as a sampling frame (N).

$$n=\frac{N}{1+N*e^2}=400$$

Where, n = the sample size of the study; N = total number of smallholders and collectors in each peasant association/kebele; e = maximum variability or margin of error of 5% (0.05); 1 = the probability of the event occurring. Therefore, a total of 400 study participants were selected at 5% standard error with a 95% confidence interval.

Data collection

Pre-tested structured questionnaires initially designed and developed in English and translated into the local language (Afan Oromo) were used and then back to English to check for consistency and clarity. Then, face-to-face interviews were carried out about hygienic raw milk handling practices, housing conditions, milk handling practices, milk collection, milk marketing, transportation systems, and types of utensils used for milking and storage and transportation of milk. Both qualitative and quantitative data and structured and semi-structured questionnaires were used. About 18 key informants (veterinarians, para veterinarians (agricultural development agents), and heads of districts' livestock development agencies) were selected for in-depth interviews using the purposive sampling technique based on their rich experience.

Sample collection and processing

Three peasant associations were purposively selected from each study district based on dairy production potential, milk

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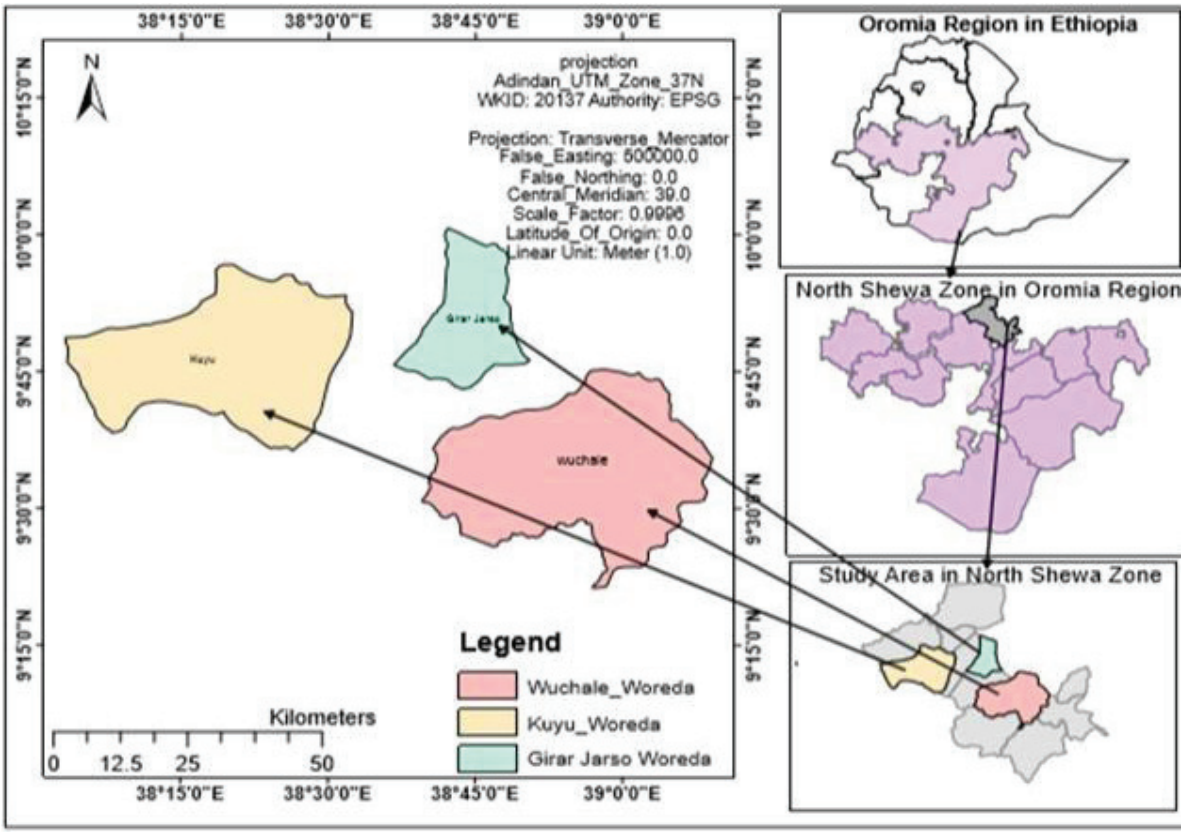

Figure 1: Map of the study areas [8].

market, access to milk supply, and collection center. Moreover, collection centers, informal merchants, and dairy cooperative unions in each district were included and interviewed in the survey. A total of 50 milk samples were collected; during collection, 254 ml of raw milk samples were collected aseptically in sterile sample bottles from milking equipment at the household level and bucket at the collection unit in a cold icebox (4°C) to restrict microbial growth and multiplication. The sample was transported to Food Microbiology Laboratory, Ethiopian Public Health Institute, Addis Ababa, for microbiological analysis as early as possible within four hours of collection. Then, serial dilutions of the milk samples were prepared soon following the standard procedures [12].

Microbial identification and enumeration

Microbial quality tests were conducted for the enumeration of bacterial and fungal loads from raw cow milk samples using Standard Plate Count (SPC), Total Aerobic Mesophilic Bacterial Count, Total Coliform Counts, spore-forming bacteria, yeast, and mold using appropriate media [12].

Total Aerobic Mesophilic Bacterial Count (TAMBC): 254 ml of milk sample collected from each dairy farmer and collection unit was dispensed into sterile test tubes in 0.1% peptone water (Himedia), as a 1:9 portion of the peptone water for initial dilution. A serial dilution was made by transferring 1ml of the previous dilution to 10^{-7}. A plate count agar (Himedia) media was used to grow the bacteria at an incubation temperature of 30°C for 72 hours in an inverted direction and the colonies were counted using a colony counter [12].

Total Coliform Count (TCC): 254 ml of milk sample collected from each dairy farmer and the collection unit was dispensed into sterile test tubes containing 9 ml of 0.1% peptone water and thoroughly mixed using a whirling mixer. Subsequent serial decimal dilutions were prepared in a similar manner using 0.1% peptone water. Duplicate appropriate decimal dilutions were surface plated on Violet Red Bile Agar (VRBA) (Himedia, India) and incubated at 45°C for 24 hours. After complete incubation, typically dark red colonies on uncrowned plates were considered Coliform for colony counts [13]. This was followed by a confirmatory test by transferring four to five colonies from each spread plate to tubes of Lactose Broth (LB) (Pharma, US) with inverted Durham tubes. Gas production after 24 hours of incubation at 32°C was considered sufficient evidence of the presence of coliform [12].

***Staphylococci aureus* Count (SaC):** Two ml of milk sample was thoroughly diluted, mixed, and plated on Mannitol Salt Agar (MSA) plates. The dried plates were incubated for 45 - 48 hours at 35°C. Typical Staphylococci colonies appeared as golden yellow, smooth, circular, convex, and moist were counted. For confirmation, a catalase test, coagulase test, and DNase test were conducted and, finally, hemolysis was detected [12].

E. coli* Count *(ECC): Ten ml of raw cow milk was homogenized in a sterile sample container with saline solution. Decimal dilutions were prepared (10^{-1} to 10^{-6}). Each dilution was used to inoculate MacConkey agar using a spread plate method. Plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for suspicious colonies of *E. coli.* Pink to

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red colonies was suspicious of *E. coli*. For further confirmation, the indole test was conducted as follows: suspicious colonies were cultured on the nutrient broth and incubated for 24 hours at 37°C. Then, 0.3 ml of Kovac's reagent was added and checked for dark red color at the top of the test tubes; which are peculiar characteristics of a generic *E. coli* [12]. Finally, the pure colonies were carefully enumerated.

Yeast and Mold Count (YMC): Samples of raw milk were diluted following similar methods as for aerobic plate count (APC) but dilutions were surface plated on potato dextrose agar at a pH of 5.6±0.2. The dried plates were incubated at 25°C for seven days. Colonies with a yellow-white and slightly dark color were counted as yeasts and molds [12].

Data management and analysis

The data entry, organization, and summarization were performed on an excel spreadsheet (Microsoft office® excel 2007). For the analysis, SPSS version 23 (Armonk, NY: IBM Corp) software was used. Descriptive statistics were employed to describe the frequency, mean values, and proportion (percentages) of respondents; the microbial loads were calculated using a logarithm function. A p-value of < 0.05 and a 95% confidence interval were used to determine the statistical significance of an estimate.

Results

Housing and cleaning practices

In the present study, the majority (79.8%) of the study participants were keeping their cows under better husbandry practices like housing, health care, and barn hygiene; 84.5% of whom were providing a separate house to their dairy cow. For 72% of the study participants, the dairy cows' barn floors were the earthen floor, while 18.5% of them constructed a barn with a concrete floor. Also, 49.2% of the study participants had the practice of daily cleaning their dairy cow barn (Table 1).

Hygienic milking and milk utensils hygiene

The majority of the respondents (75.8%), have replied that milkers wash hands before milking and between milking two or more cows; however, the remaining 24.2% of them did not practice hand washing both before and between milking. On the other hand, 64% of the respondents have the trend of washing cow's udder before milking; while 34% of them didn't wash cow udders. The study has also shown, that 5.3% of the respondents practice teat dipping using antiseptics at the time of milking, while 94.7% did not. For cleaning milk utensils; 45.5%, 28.7%, and 26.8% of the respondents used river, pond, and pipeline water, respectively. Respondents also indicated that there was a trend of smoking milk utensils using olive leaf and stem; among which 47% of them fumigate the milk utensils before and after use (Table 2).

Milk marketing and transportation

The present study has revealed that the majority (72.2%) of the respondents sold milk to milk processors and collectors.

Table 1: Dairy cow housing and cleaning practices in North Shewa zone, Oromia, Ethiopia.

Variables		No	Percent (%)
Availability of dairy cattle housing and facilities	Yes	319	79.8
	No	81	20.2
Types of barn	Separate house	338	84.5
	Fenced barn	59	14.8
	Common house with humans	3	0.7
Hygienic status of the barn floor	Concrete	74	18.5
	Cleaned floor	38	9.5
	Muddy Soil floor	288	72
Frequency of cow's barn cleaning	Daily	197	49.2
	2-3 times a week	108	27.0
	Once a week	85	21.8

Table 2: Hygienic practices of milkers and milk utensils hygiene in North Shewa zone, Oromia, Ethiopia.

Variables		No	Percent (%)
Milkers wash hands before milking and between milking	Yes	303	75.8
	No	97	24.2
Washing cow's udder before milking	Yes	256	64
	No	144	34
Teat dipping	Yes	21	5.30
	No	379	94.7
Water used for cleaning milk utensils	Cold	319	79.8
	Warm	63	15.7
	Both	18	4.54
Sources of water for cleaning milk utensils	Tap	107	26.8
	Pond	111	28.7
	River	182	45.5
Frequency of cleaning milk utensils	Before milking	296	74
	After milking	96	24
	Before and between milking	8	2
Smoking of milk utensils	Yes	188	47.0
	No	212	53.0

Nearly half of the respondents (48.3%) sold milk due to a lack of modern milk processing facilities. Most of the respondents (76.5%) used plastic containers, while the rest 23.5% of them used aluminum-coated metallic utensils for holding and transporting milk. The majority (88%) of the respondents used foot transportation to supply milk for collectors in the study areas (Table 3). This situation is similar to the location in the study areas.

Microbial quality of raw cow milk

The study has revealed the load of EcC as 20% at households and 12% at collection spots; whereas SaC it has shown a microbial load of 20% and 6% at households and collection spots respectively. Table 4 below shows the microbial load test values of raw cow milk evaluated during the study.

The mean values of TAMBC in the study were 4.391±0.15 for households and 4.836±0.206 for collection spots with an overall mean of 4.513±0.170. The TAMBC were 3.762±0.161 log10 CFU mL^{-1} at the household level and 5.721±0.055 log10 CFU mL^{-1} at collection spots with the overall mean of 4.188±0.166 log10 CFU mL^{-1}. There was a statistically significant difference between

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TCC and the sources of milk ($p<0.05$). In this study, the mean values of EcC were 3.343±0.122 for households and 5.414±0.100 for collection spots with an overall mean of 3.573±0.133. The study has also shown EcC mL^{-1} of milk samples collected from household milk producers was lower than the raw milk samples obtained from collection spots; however, the difference in EcC between the sources of milk was not statistically significant ($p>0.05$). The mean values of SaC were 2.470±0.038 for household milk producers and 2.249±0.093 for collection spots with an overall mean of 2.396±0.059. It has been connoted that SaC per milliliter of milk samples collected from household milk producers was higher than the raw milk samples obtained from collection spots, but the difference was not statistically significant ($p>0.05$). Similarly, the mean values of YC were 2.944±0.074 for household milk producers and 3.372±0.091 for collection spots with an overall mean of 3.106±0.082. The mean values of MC were also 2.479±0.039 for household milk producers and 2.670±0.037 for collection spots with an overall mean of 2.577±0.038. However, there was no statistically significant difference between MC and the sources of milk ($p>0.05$) (Table 5).

Discussion

The study was the 1st of its kind to analyze the microbial quality and handling practices of raw cow milk in the milk belt areas of the north Shewa zone, Oromia, Ethiopia. Both qualitative and quantitative research methods were employed to perform the study. Poor housing conditions, the unhygienic and poor health status of the lactating cow, unhygienic milking methods, milk storage, and transportation equipment, and unclean water used for washing milk equipment were identified as the major contributing factors to the contamination of raw cow milk. This finding was in line with the finding of Gurmessa [14,15] and Amenu et al. [16]; which identified the health status of a lactating cow and, unclean water and milking utensils as the most decerning factors which deteriorate the microbial quality of raw cow milk. It was also analogous to other findings that were conducted in the central highland of Ethiopia [17,18]. However, it disagrees with the finding of Sintayehu and Haile who reported the highest microbial load of 96.2% from traditionally fermented milk-'Irgo', collected from Hawassa town, southern Ethiopia [3].

Similarly, the result of the present study has shown that the mean values of TAMBC and Staphylococcus aureus were higher at the household level as compared to the collection spots; 6.30 and 5.30 log10 CFU mL^{-1}, respectively. This in turn is lower than the upper acceptable limit given by the East African Community Standard [19]. However, it was lower than the findings of Amentie et al. [18] in rural areas of Babile district, eastern Ethiopia. This difference might be due to inadequate dairy infrastructure and lack of vehicles with cooling systems coupled with limited knowledge of the hygienic milk handling practices [15,20,21].

The study was also shown the higher TCC and EcC of raw cow milk obtained from collection spots than at the household level regardless of the location of the study areas. This finding also agrees with the finding of [20,22-25] which declared the higher microbial load of raw cow milk obtained from the bulk tank. It also agrees with the finding

Table 3: Milk marketing and transportation in North Shewa zone, Oromia, Ethiopia.

Variables		No	Percent (%)
Where do you sell your milk	Milk processors and cooperatives	289	72.2
	Individual collectors	65	16.3
	Cafés, restaurants, and hotels	46	11.5
Reason for sale at these places	Absence of a modern processor	193	48.3
	Presence of collection units	94	23.5
	High demand	69	17.2
	Surplus product	47	11.0
Milk transporting utensils	Plastic	306	76.5
	Aluminum coated metal	94	23.5
Means of transportation	On foot	352	88
	Horse cart	36	9
	Public transport	12	3

Table 4: Microbial test values of total cell counts, *E.coli* counts, *Staphylococcus aurous* counts, yeast and mould counts from raw cow milk in North Shewa zone, Oromia, Ethiopia.

Sources of Milk Samples				
Isolated Microbes		Households	Collection Spots	
TAMBC	Yes	24 (48%)	7 (14%)	31 (62%)
	No	14 (28%)	5 (10%)	19 (38%)
TCC	Yes	18 (36%)	5(10%)	23 (46%)
	No	20 (40%)	7 (14%)	27 (54%)
Yes		10 (20%)	6 (12%)	16 (32%)
EcC	No	23 (46%)	11 (22%)	34 (68%)
SaC	Yes	10 (20%)	3 (6%)	13 (26%)
	No	27 (54%)	10 (20%)	37 (74%)
YC	Yes	18 (36%)	10 (20%)	28 (56%)
	No	20 (40%)	2 (4%)	22 (44%)
MC	Yes	9 (18%)	8 (16%)	17 (34%)
	No	28 (56%)	5 (10%)	33 (66%)

Table 5: Mean±SD of microbial counts log10 CFU mL^{-1} of raw cow's milk samples from milk collectors and household level in North Shewa zone, Oromia, Ethiopia

	Souces of Milk Samples			
Variables	Households	Collection Spots	Overall (Mean±SD)	*P-values*
Total Aerobic Mesophilic Bacterial Count (TAMBC)	4.391±0.158	4.836±0.206	4.513±0.170	0.517
Total Coliform Count (TCC)	3.762±0.161	5.721±0.055	4.188±0.166	0.015
E.coli Count (EcC)	3.343±0.122	5.414±0.100	3.573±0.133	0.153
Staphylococcus aurous (SaC)	2.470±0.038	2.249±0.093	2.396±0.059	0.518
Yeast count (YC)	2.944±0.074	3.372±0.091	3.106±0.082	0.175
Mold count (MC)	2.479±0.039	2.670±0.037	2.577±0.038	0.238

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of Gebretsadik et al. [26] who reported a significantly lower proportion of contamination of milk collected from dairy farms as compared to milk from vendors and cafeterias. This might be due to cross-contamination of milk during transportation, poor environmental sanitation, lack of sanitation of storage containers, and lack of temperature control throughout the value chain. In addition, since the raw milk samples obtained from collection spots were taken from the bulk tank, contaminated milk from a single household may deteriorate the microbial quality of the entire milk in the tank; and hence, increase the microbial load of the milk samples obtained from collection spots. Thus, it is logical to see increased microbial load in the milk sample obtained from collection spots. The mean values of yeast and mold counts were also higher at collection spots than at the households' level which was higher than the upper acceptable limit of 2.1 and 1.7 log10 $FUmL^{-1}$, respectively [16]. This was also in agreement with other findings that were conducted in the eastern highland of Ethiopia [19,27-29]. The presence of high numbers of yeast and mold in milk indicates that the milk has been contaminated with soil, specks of dust, air, and other contaminants due to poor handling practices of raw milk. Moreover, milk was collected on dusty roadsides without shade and cooling systems in study areas.

The result of the key informant interview was triangulated with qualitative data on why most milk producers and collectors used equipment made up of plastic materials and poor milk handling practices in the study areas. This result was in line with the finding of Amenu et al. [16] in southern Ethiopia.

Taken all together, the study indicated a knowledge gap in hygienic handling practices of raw cow milk and a higher microbial load of milk collected from collection spots as compared to milk collected from households. However, no significant difference was observed either in the knowledge gap of dairy cow producers or microbial load over locations in the study areas.

Limitation of the study

The scope of the study was limited to the three districts so the study couldn't represent the whole North Shewa zone. A cross-sectional study design was employed in this study it is impossible to see a causal relationship by using this design [21].

Conclusion

The microbial analysis indicated that the mean values of TAMBC and SaC were higher at the household level than at collection spots, and both were lower than the upper acceptable limit. However; the mean values of TCC, EcC, YC, and MC were higher at collection spots than at the household level. The study has also shown poor handling practices of raw cow milk, which was primarily due to inadequate dairy infrastructure and limited knowledge on the hygienic handling practices of raw cow milk. Therefore, awareness should be created among milk producers and collectors concerning hygienic handling practices of raw cow milk. Moreover, milk collectors should use a cooling system to maintain bulk tank temperature to limit microbial load and subsequent milk spoilage and consumer safety.

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