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Assessment of Bacteriological Quality Major Bacterial Pathogens and Handling Practices of Raw Camel Milk in Ab'ala Woreda of Afar National Regional State, Ethiopia

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Abstract

In Ethiopia, most of the camel milk production is accounted by the traditional milk processing system and unhygienic handling that are generally poor in processing capacity, causing high product loss and risky for public consumption. A cross-sectional study was carried out in Ab'ala woreda of Afar national regional state from August to November 2017 with the objective of assessing the bacteriological quality, handling practice and major bacterial pathogens of camel milk. The study methodology employed was questionnaire survey and observational study for handling practice and bacteriological count and isolation and detection of major pathogenic bacteria from raw camel milk. Thirty (30) purposively selected camel herders were interviewed for the survey-based study of milk handling practices and forty raw milk samples were aseptically collected and tested for bacteriological load analysis and isolation of major bacteria. The overall average total bacterial count ranged from $2.3x10^9$ to $1.65x10^9$ cfu/ml with a mean value of $56.20x10^9$ milk samples directly from the teat and $92.25x10^9$cfu/ml milk samples from equipment (buckets) at farm level. Results showed very significant differences in total plate counts ($P < 0.05$) between the two milk collection points. The total bacterial count of the milk samples was high in buckets when compared to the samples taken from the teat directly. In this study milking system and handling practices like udder cleaning, hand washing before milking and cleaning vessels was in poor sanitary condition. No statistically significant variation was observed in coliform counts in milk samples collected from the teat compared to milk samples from the equipment used to milk camels. Meanwhile, coliform counts demonstrated a limited increase ($P > 0.05$) from udder (teat) to equipment (bucket) level. High coliform count in milk indicates fecal contamination of milk. Milk was produced and handled in unhygienic condition. The results of the current study indicated that the camel milk produced and handling in the study area can generally be considered as substandard in quality for consumption unless pasteurized. Therefore, this risk assessment study with similar different studies reported from different regions in Ethiopia might provide a basis for the establishment of national milk quality standards in Ethiopia.

Article Info

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Keywords

Ab'ala; Bacterial quality; Camel milk; Ethiopia; Handling practice

Introduction

The camel is belonging to *camelidae* family of mammals, order of *Artiodactyles* and to the sub-order of *tylopoda* (animals with padded feet). The large *camelids* are represented by two domesticated species, the one-humped camel (*dromedary*) and the two-humped camel (*Bactrian* camel), One humped camel found in the hot arid lands from North and eastern of Africa and eastern part of Asia and the two humped camels found in cold steppes and deserts in Central Asia (Ji and Meng, 2009). The domestic camel now inhabits the deserts of northern

and north-eastern Africa, the Middle East and central Asia (Lever, 2009). According to FAO (2010), there are about 25.3 million camels in the world, Somalia with 7 million heads has the largest camel population in the world followed by Sudan with 4.5 million. Among other domestic ruminant, camels has the ability to produce milk of good composition and quantity for human consumption even during dry seasons and drought years when milk from cattle, sheep and goats is scarce (WHO; 2014). Oliver *et al.,* (2009), stated that camel's feeding behavior, tolerance to high salt contents and ability to conserve water, make it the best of ruminants for arid and many semi-arid area. The chemical composition of camel milk is relatively similar to that of cow milk. The camel milk composition ranges are: 9.08-14.4% dry matter, 2.7-4.5% protein, 3.2-5.5% fat 4.0-5.6% lactose and 0.63-0.9% ash (Farah and Fischer, 2004).

Ethiopia hosts about 2.1 million heads of camel which are found in the arid and semi-arid regions of the country, this number ranks the country third in Africa after Somalia and the Sudan and fourth in the world (FAO, 2010). Livestock represents major national resources and form an integral part of agricultural production system in Ethiopia (Gebrewold *et al.,* 2000). There is a rapid increasing demand for livestock products in developing countries as a result of population and income growth as well as urbanization (Delgado *et al.,* 1999). Annual milk consumption increase in these countries averaged 3.5 to 4.0% between 1995 and 2005 (FAO, 2010). In Ethiopia around 97% of the annual milk production is accounted by the traditional milk processing system using on-farm traditional milk processing materials (Felleke, 2003). Milk is an excellent culture medium for the growth of microorganisms. The rate of multiplication of microbes depends mainly on storage temperature and time, level of nutrients and handling conditions. Consumption of raw milk combined lack of cold facilities, high temperature and low hygienic condition of milking personnel and equipment are the major factors responsible for illnesses caused by foodborne pathogens as numerous epidemiological reports have implicated (Musinga *et al.,* 2008).

Bacterial food poisoning is among the most prevalent causes of gastroenteritis worldwide (European Food Safety Authority, 2010; CDC, 2016). Each year 1.8 million people die as a result of diarrheal diseases and most of these cases are attributed to contaminated food or water. Improper food preparation can expose to most food-borne diseases. More than 200 known diseases are transmitted through food (WHO, 2014). Numerous epidemiological reports have implicated non-heat treated milk and raw-milk products as the major factors responsible for illnesses caused by food-borne pathogens (Ahmed *et al.,* 2010). Raw camel milk contain microorganisms pathogenic for man and the contamination can generally occur from three main sources; within the udder, outside the udder, and from the surface of equipment used for milk handling and storage (Vahedi and Nasrolahei, 2011).

Globally, most of camel milk is consumed in the raw state without any heat treatments or acid fermentation and kept at high ambient temperature coupled with lack of refrigeration facilities, unclean of milker's hands during milking and transporting. These conditions turn the milk to be unsafe, capable of causing food-borne diseases and it even spoil fast, (Benkerroum *et al.*, 2003). Unhygienic handling practices in traditional camel milk production, unclean utensils and lack of processing and less knowledge of camel farmers about camel health management and associated risk factors about the consumption of raw camel milk are major challenges of camel production (Siboukeur, 2007). In Ethiopia 98% of the camel's milk are consumed in the raw state without any heat treatments (Eyassu and Mehari, 2007). Taken together, the present state of milking and milk handling practice may pose health risks to the public. These risks are linked to contamination of milk, growth and survival of harmful pathogens in the milk and increasing number of other micro-organisms caused by poor handling and conditions such as temperature and humidity.

Problem statement and justification of the study

Camel milk is the key foods for pastoralists and pre-urban in the arid and semi-arid areas of eastern lowlands of Ethiopia where browse and water are limited (Felleke, 2003). In Ethiopia as whole and specially the study area camel milk are mostly consumed raw without heat and refrigeration or chemical treatments. In the traditional sector there is evidence of inappropriate milking and poor handling of milk, which predispose milk to bacterial contamination. Furthermore, because of the greater prevalence of tropical diseases among livestock in the traditional sector, lactating and milking animals might have inborn pathogens in blood. These may shed harmful pathogens in milk and negatively affect the health of consumers of milk or milk products.

Preliminary results obtained in recent studies of Abera *et al.,* (2016*)* have shown that high number of bacterial load and harmful pathogens exist in camel milk of Fafen

zone, Ethiopian Somali regional. However, in Afar region there is a limited studies on camel milk bacterial quality, it was therefore worthy carrying out a study that will fill this information gap. Hence, this study is intended to address the shortcomings in the study area with the following objectives.

General objective

To assess the bacteriological quality, major milk bacterial pathogens and handling practice of raw camel milk in Ab'ala woreda of Afar national regional state

Specific objectives

To evaluate the bacteriological quality of raw camel milk in the study area

To study the milking and milk handling practice of camel milk producers of the study area

To identify the major bacterial pathogens in raw camel milk

Materials and Methods

Study area

The current study was conducted in Ab'ala woreda of Afar national regional state. Ab'ala is located in the northern part of Afar region. The woreda lies approximately between 13°15' and13°30' North latitude and 39°39' and 39°55' East longitude. It is about 50 km east of Mekelle city, Tigray region.

The woreda is characterized by a semi-arid type of climate receiving a bimodal rainfall on average about 422 mm. The soils are generally sandy and salty (CSA, 2007).

Ab'ala is bordered on the south by Megale, north by Berhale, northeast by Afdera, and east by Erebti woredas and on the west by the Tigray Regional State. About 27%, 10,301 (2,396 households) of the total population of the woreda are urban inhabitants.

The rest 73%, 27,662 (4482 households) lives in the rural area. Livelihood of the people in the woreda is dependent on livestock production. The livestock population in the woreda is estimated at 33,938 cattle, 34,144 heads of sheep, 149,450 heads of goats, 32,069 camels and 725 mules (CSA, 2007).

Study population

Lactating she-camels were the target animals for the milk samples and the camel herders were the source of the data during the questionnaire survey.

Study design

A cross-sectional study was carried out from August to November 2017 in Ab'ala woreda of Afar national regional state, for assessment of the bacteriological quality, handling practice and identification of major pathogenic bacteria in the raw milk.

Sampling strategy

Ab'ala district was purposively selected for this study because of the camel population and its surrounding pastoral community who rear mainly camels. The district has thirteen kebeles, of these kebeles three kebeles had being selected purposively due to camel population, willingness to precipitate and visibility of infrastructures. From each of the selected Kebeles, ten households was randomly selected and thus a total of 30 households who rear camels was interviewed using a semi-structured questionnaire applying a double-visit multiple-subject diagnostic survey technique.

Data collection methods

Observational study

Observations were undertaken focusing on pastoralists who own camels and produce camel milk, in order to get background information regarding production system, handling and utilization practices of camel milk in the area. The status of camel rearing and health management practices, hygienic and safety status of the production and post-harvesting loss of camel's milk, risk factors associated with camel's milk (raw)spoilage and consumption of the community etc. were collected by well regulated field checklists during the planned visits to the study areas. All events observed during the personal observations was recorded on respective formats prepared for each specific activity.

Questionnaire survey

Semi-structured questionnaires (Appendix 1) were used to assess the hygienic and handling practices of camel milk. Thirty milking personnel and family members who are involved in camel milk production and utilization

were selected and interviewed. Consequently, hygienic practices employed in the study areas such as house cleaning, udder cleaning, hand washing practices and milking utensils and collecting vessels (buckets) hygiene and other conditions thought to affect the hygienic quality of raw milk were assessed. Routine mastitis control practices, knowledge on health risks associated with consumption of raw milk, knowledge of factors affecting hygiene or quality of camel milk were also performed. The questioners were administered by face-to face interviewing.

Laboratory investigation

Milk collection and transportation

A total of 40 raw camel milk were collected. Twenty of the raw camel milk was directly collected from the udder. The teats were cleaned and dried before sampling; each teat end was scrubbed gently with cotton swabs moistened with 70% ethyl alcohol. The first 3–4 streams of milk were discarded, and approximately 250 ml of milk was collected into sterile sampling bottles and 20 milk samples were collected from traditional milking buckets. Each specimen was labelled and placed in ice box and transported to Veterinary microbiology laboratory, Mekelle University. After arrival at the laboratory, samples were preserved in refrigerator at +4 °C temporarily for 24 h for processing.

Bacteriological Analysis of milk

The collected milk samples were analyzed for determination of bacterial quality. The bacteriological tests considered for enumerations of the bacterial load in raw milk samples were total bacterial count (TBC) and coliform count (CC). For these two procedures standard plate count agar (Oxoid, UK) and violet red bile agar (HiMedia, India) were used, respectively. Peptone water was used for serial seven-fold dilutions.

Total plate count

For total plate count demonstration, one ml raw milk was transferred from each sample to 9 ml sterile peptone water (15%) and thoroughly mixed to give 1:10 dilution. Serial dilutions were made by transferring 1 ml of the previous dilution in 9 ml of sterile distilled water up to 1:10,000 dilutions. Then only 0.1 ml sample from the 7^{th} dilution level was cultured by a glass spread method to the standard plate count agar (Oxoid, England) and one ml of the sample from appropriate decimal dilution were placed on duplicate Petri dishes. Total Bacterial Count was made by incubating cultured dilutions of milk samples on Plate Count Agar (Oxoid, England) plates. Colonies were counted after the culture media was incubated at 37 °C for 24 h. Total number of colonies on plates 30 to 300 per plates was selected and colonies were counted (Weldaragay *et al.*, 2012). Finally, colony count were made using colony counter (RDC, M671: England).

Coliform count

For coliform count, 1 ml of milk sample were serially diluted in 9 ml of peptone water and volumes of 1 ml of appropriate dilutions were plated in Petri dishes by the spread technique using Violet Red Bile Agar (VRBA) which was boiled for about 5 minutes. Typical dark red colonies were counted as coliforms after incubation at 30^{o}C for 24 hours (Richardson, 1985).

Detection of major pathogenic bacteria from camel milk

Detection of *Staphylococcus aureus*

Small amount of milk samples was placed in Manitol salt agar plates and streaked using a wire loop and incubated for 24hours. The presumptively identified *S. aureus* from mannitol salt Agar were sub cultured to nutrient agar plate and after 24 h culture colonies of *S. aureus* was picked by bacteriological loop and placed on clean slide and subjected to gram stain and observed under microscope for Gram's reaction, shape and cell arrangements. Pure culture of the isolates were picked using a sterile loop and tested for catalase on sterile glass slides. For further conformation citrate test, indole test and motility test were performed. The identification was based on the fact that *S. aureus* rapidly ferment maltose within 24 h and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. The rapid fermentation (24 h) was considered as *S. aureus* isolates (Quinn *et al.*, 2002).

Detection of *Salmonella* spp.

Salmonella spp were detected according to the procedure outlined by Food and Drug Administration (FDA, 2001). One ml of milk was suspended with 9ml of buffered peptone water and diluted up to 10^{-7} dilution. After dilution 100ml of the suspension was inoculated into salmonella shigella plates and the plates were then incubated at 37°C for 24 hours. The presumptively

identified colonies from SS agar were sub cultured to nutrient agar plate and after 24 h culture colonies were picked by bacteriological loop and placed on clean slide and subjected to Gram stain and observed under microscope for Gram's reaction, shape, cell arrangements and color.

Pure culture of the isolates were picked using a sterile loop and subjected to biochemical tests including catalase test, citrate test, indole test and motility tests. Cells of typical colonies with large, glossy black centers or that appear almost completely black colonies were identified as *Salmonella* spp.

Detection of *Escherichia coli*

E. coli was detected using Eosin methylene blue agar media which is selective for *e-coli* isolation. One ml of milk sample was added into 9ml of sterile peptone water diluents. After sufficiently homogenizing the suspension and preparing serial dilutions, 0.1 ml of the appropriately diluted sample was spread evenly onto the surface of Eosin methylene blue agar media.

The inoculated petri plates were then incubated at 35°C for 18-24 h. The presumptively identified colonies from EMB agar were sub cultured to nutrient agar plate and after 24 h culture colonies was picked by bacteriological loop and placed on clean slide and subjected to gram stain and observed under microscope for gram's reaction, shape, cell arrangements and color.

Pure culture of the isolates were picked using a sterile loop and subjected to biochemical tests including catalase test, citrate test, indole test and motility test were performed. The identification was based on the cells of typical colonies having golden color were considered for e-coli positive colonies and golden color were observed.

Data management and analysis

All data obtained through microbiological analysis were entered into Microsoft Excel spread sheet. Statistical data analysis was carried out using SPSS software (Version 20).The microbial counts were first transformed to colony forming units per milliliter of sample (log cfu /ml) and the results was presented as the geometric means and other descriptive statistics.

The CFU/ml of sample was determined using the formula CFU per ml of sample = number of colonies / (amount plated X dilution).

Results and Discussion

Socio-economic and demographic characteristics of households

The size of camel herds of the interviewed herders ranged from 5 to 40 head per household with an average of 20 camels. The production system practiced predominantly in the study area is extensive pastoral type. The pastoral/agro-pastoral communities of the study districts keep mixed herds mainly cattle, sheep, goat and camel with variable proportion mainly based on availability of grazing pasture and browsing plants. About 89% of the camel herds of the surveyed households were female comprised of lactating camel (40%), followed by dry camels (31%), (18%) heifers and female camel calve and (11%) bull camel and male calves during the study period. The number of female camels far exceeds male camels to ensure reproductive potential and sufficient milk production which is main source of food for pastoralists of the district. Of the total 30 sample surveyed household heads from the camel milk producers, 11 (26.6%) were female headed and the remainder 19(63.4%) were male headed households. As far as age of the household heads is concerned, the average age of the sample households was 39.83 years ranged from 23 to 95 years old.

Educational level

Out of the total household heads about 20 (70%) of respondents did not attend any formal education (illiterate) and the remaining 10 (30%) household heads did attend formal education or they are literate (figure 1). This indicates that more risks are likely to occur at the herd level where the herdsmen are involved of milking and handling of milk.

Camel farmer's knowledge on causes of milk contamination

More than half of the respondents (53.33%) had no knowledge on the causes of milk contaminations and 46.66% had the knowledge on the cause of spoilage of milk. Farmers attributed milk spoilage to different factors as shown in Figure 2.

Hygienic measures, milking practices and milk handling approaches of the camel milk producers

The practices of milking and milk handling in the study area were generally in poor hygienic conditions (table 1).

Unclean hands of milkers, unclean milking utensil, unsafe water for cleaning, dirty camel udder, milking environment, milking of diseased camels, mixing of milks of healthy and diseased camels, and consumption of raw camel milk were the practices associated with milk spoilage and public hazards shown in this study. Thus, milk produced and handled under such conditions would have poor quality and may contain pathogenic microorganisms of public health concern.

Camel milk yield, milking frequency and milk consumption habits of the respondents

Hand milking was the only way of milking camels by men using a milking vessel (locally called *Ayni*) and it was observed that milking was done after the calf was suckled the dam for a few minutes to stimulate milk let down.

The milking frequency in the study area was different (Table 2) depending on the season, feed availability and lactation stage of the animal. Morning milking was done between 6:00-7:00AM, noontime milking was 11:00am-12:30PM mostly held in watering points and evening milking was carried out at 5:00-6:00PM. Camel milk yield depends on several factors including feed availability, season and lactation stage. Majority of the respondents preferred camel milk than milks of other species in the study areas and milk was mainly consumed in its raw state without being subjected to any sort of treatment like boiling and fermenting as indicated in table 2 below.

Camel milk marketing involvements and service access to wards milk productivity and utilization

Though demand for milk marketing and processing exists, camel milk marketing and optimized fermentation were not practiced in the study areas (Table 3).Furthermore, there is no information access about camel milk processing and marketing opportunities. There is also lack of material and technical supports for the camel milk producers. (Table 3).

Bacteriological quality analysis of camel milk

Total bacterial count

The total bacterial counts demonstrated in the laboratory revealed that milk samples from buckets had significantly higher mean TBC ($P < 0.05$) as compared to that of milk samples directly taken from the teat. Similarly, mean TBC showed a statistically significant ($P < 0.05$) increase from teat to container (bucket) level (Table 4).

Coliform counts

No statistically significant variation was observed in CC in milk samples collected from the two districts. Meanwhile, CC demonstrated a limited increase ($P > 0.05$) from udder to equipment level (Table 5).

Major bacterial pathogens in raw camel milk

Camel milk used in this study was found containing Gram negative bacteria (52.5%) and comparable amount of Gram positive bacteria (47.5%) in (Table 3). Furthermore, *Staphylococcus* spp., *E. coli*, and *Salmonella* spp. Have been isolated from raw camel milk at different levels as in dictated in figure below (Fig 4). *Staphylococcus* spp. showed the highest prevalence at production level whereas that of coliforms tends to increase from production to bucket level (figure 4).

Camel milk is a key food for pastoralists in the arid and semi-arid areas of eastern lowlands of Ethiopia. It is traditionally prized for its anti-oxidant, anti-cancer, anti-diabetic and more generally as restorative properties in convalescent patients.

Milk has been identified as a vehicle of several organisms in many occasions. The poor hygienic production of milk is more likely to cause milk-borne diseases and the natural antimicrobial factors can only provide a limited protection against specific pathogens for a short period. Such risk is higher when the milk is consumed in its raw state as is commonly practiced by the local producers Mohammed *et al.*, (2016). The production system of the Afar region is dominated by pastoralism (90%) from which agro-pastoralism (10%) (MARD, 2008).

The milking practice and different vessels used for milking and storage of camel milk in the present study are indicated in (Table 1). The source of contamination of the milk can be from the milker's hand, vessels used for milking and milk storage and poor sanitation of the udder of the lactating camel. These are the preconditions in production of quality milk and milk products. As indicated in (Table. 1), half (50%) of camel household heads showed that they wash hands before milking to keep the hygiene of the milk and rest (50%) did not practice hand washing before milking.

Table.1 Milking practice and hygienic measures of the surveyed households

Factor	Category	Number of respondents	Percent%
Hand washing before milking	Yes	15	50
	No	15	50
Source of water	Streams	17	56.6
	Well water	9	30
	Tap water	4	13.4
Where do you milk camels	Open air	30	100
Method of cleaning milk containers	Washing and smoking	20	80
	No	10	20
Types of plants used for smoking	Bieresa, Leideno and Alibal	10	20
	Leideno, Beiresa and Jirimme	20	80
Purpose of smoking milk containers	To prevent milk from spoilage	20	80
	Increases milk platabitilty	10	20
Udder/Teat washing before milking	Yes	0	0
	No	30	100
Milking container washing before milking	Yes	22	73.33
	No	8	26.66
Milking container washing after milking	Yes	19	63.33
	No	11	36.66
Do you milk diseased camels	Yes	5	16.6
	No	25	83.4
Milking order	Sequentially	4	13.33
	Randomly	26	86.66
	Clay pots	5	16.66
Materials for milk storage	Plastic jerry cans	19	63.33
	Hide	6	20
	Fiber made (Locally made)	30	100
Vessels for milking	Clay made		
Market linkage and information access (100%)	No	30 + 9 consumers	(100%)
Milk storage Utensil support	No	30	100%
Information access about milk fermentation Methods	No	30	100%

Table.2 Camel milk yield, milking frequencies and milk consumption habits

Factor	Category	Number of respondents	Percent	Remark
Milking frequency	One time	6	20	Depends on lactating stage and season
	Two times	17	56.7	
	Three times	7	23.3	
Milk yield/camel/day	2-3 liter	11	36.6	
	4-6 liter	15	50	
	6-8 liter	4	13.4	Depends on lactating stage and season
consumption pattern	Raw	27	90	
	Slightly fermented	3	10	Include consumers
	Boiled	0	0	
Milk preference	Camel milk	31	79.5	Producers/consumers
	Cow milk	4	10.25	
	Goat milk	4	10.25	

Table.3 Camel milk marketing involvements and related service accesses

Factor	Category	Number of respondents	Percent
Willingness to sell camel milk	Yes	9	30
	No	21	70
Willingness to buy camel milk (consumers)	Yes	7	77.7
	No	2	22.3
Information access about milk related health problems (producers and consumers)	Yes	11	28.2
	No	28	71.8
Information about drug withdrawal period (Both for producers and consumers)	Yes	15	38.5
	No	24	61.5

Table.4 Mean values of total bacterial counts from different sources

Parameter	No of samples	Min	Max	Mean TBC (CFU/ml)	P value
Sampling level					
Udder (teat)	20	$2.3x10^9$	$9.2x10^9$	5.62x10	.000
Milking bucket	20	$4.9x10^9$	$1.65x10^9$	$92.25x10^9$	

Table.5 Mean values of coliform counts from different sources

Parameter	No of samples	Min	Max	Mean CC (CFU/ ml)	P value
Sampling levels					
Udder	20	$1.6x10^5$	$9.8x10^5$	$4.4x10^5$	.164
Buckets	20	$1.8x10^5$	$1.3x10^5$	$6.1x10^5$	

Fig.1 Educational background of the respondents

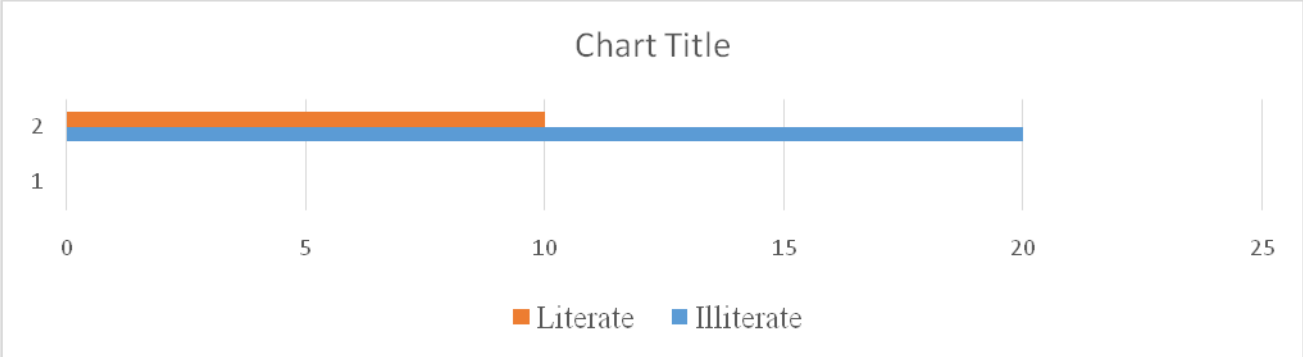


Fig.2 Causes of milk contamination as reflected by the respondents

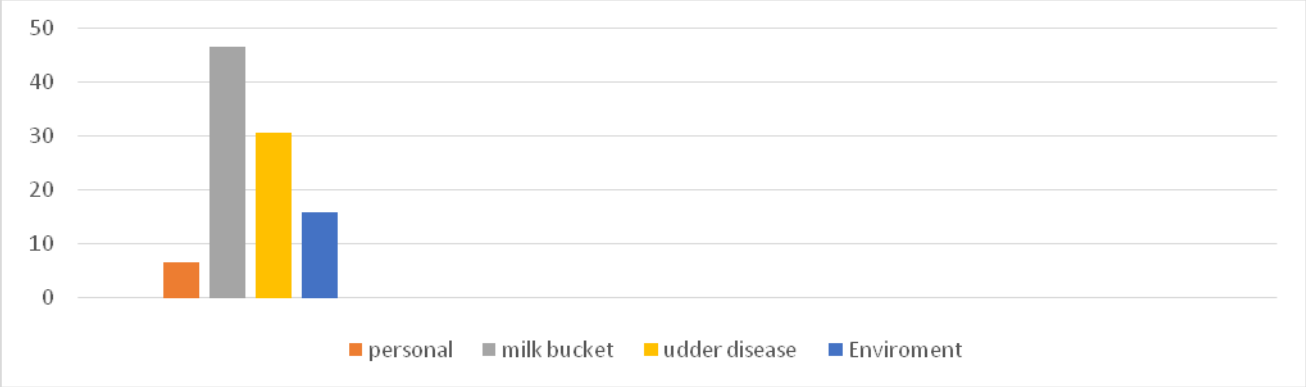


Fig.3 Percentage of gram negative and gram positive bacteria

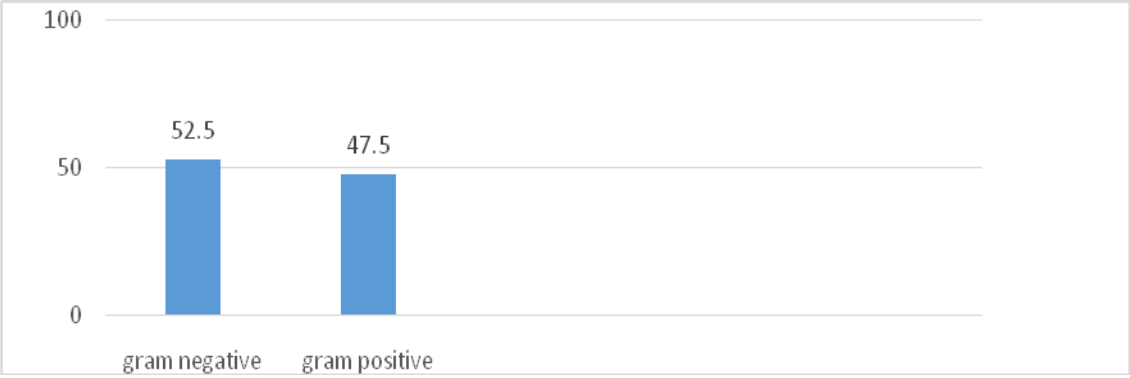
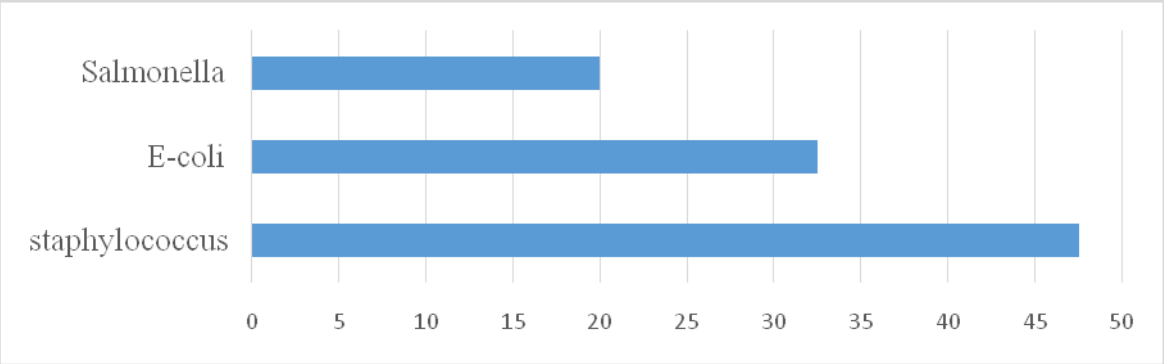


Fig.4 Bacterial isolated from milk samples



As per the belief of the pastoralists of the study area, hot or cold water utilization for washing the udder of she-camel either before or after milking can cause an early dry off of the lactating camel and water cannot be used for cleaning the udder (Table 1). This is in agreement with Muli *et al.*, (2008) who reported that unhygienic milk production was the major problem for pastoralists of Isiolo district in Kenya which was caused because of unhygienic milking and handling of milk.

According to the respondents, smoking is used to prevent the milk from spoilage and to make its flavor attractive. Although same respondent used washing and formicating with plants, the milk buckets in the study area was generally unhygienic. Other studies have also reported on the use of smoke fumigation in the pastoral system (Wayua *et al.*, 2009; Kipsang, 2011). Source of water used to wash hands and equipment in the study area was different. Of the respondents (56.6%) of them used stream for washing of equipment and personal hygiene, 9(30%) use well water and 4(13.4%) tap water. Moreover very close observation of the area and their practices showed that lack of availability of clean water for washing and the unsuitability of the milk containers had resulted in the poor hygienic condition of the milk. This result is in line with the work of Lumadede *et al.*, (2010) who reported that the use of unclean plastic Jerry cans dominates the milk storage in Dollo, Somali region of Ethiopia. This might be a contributing factor for the rapid spoilage of milk, as plastic Jerry cans cannot be cleaned.

The amount of milk produced by the animal and frequency of milking can differ according to the season and lactation period of the camel. The camel herders revealed that, the milk yield and milking frequency varies mainly among seasons and lactation stage., During the rainy season, because of the availability of feed and water, camels can be milked three or four times a day; two times at day time (around 6:00 AM and around 10:00 AM) and two times at night (around 6:00 PM and 10:00 PM) while in the dry season they are milked once, twice or thrice a day (before grazing and after they come back from grazing and the mid-day at watering points). The average amount of milk obtained per camel in the study area was 4 liters per day. This result is similar with the result of Williamson, (2003) in Somalia, Spencer, (2009) in Kenya, and Abera *et al., (2016)* who reported that Somali, Ethiopian, Kenyan camels are milked in early morning, mid-day in the watering points and late afternoon and produce 5 to 12 liters of milk per day.

There are potential hazards associated with consumption of raw camel milk (Farah *et al.*, 2007). All of the respondents in the study area consume camel milk in its raw state without being subjected to any sort of processing treatment or fermentations. This is due to the cultural believes and less knowledge about milk related health problems and consumption of raw camel milk should be of major concern from public health point of view. This findings is in line with Mohammed *et al.*, (2016) who reported that 100 % of the respondents in Afar national regional sate, involved in the production reported milk is never boiled and fermented for cultural reasons and they believed that camel milk had medicinal value when drank in raw state. Due to this belief, any heat treatment of milk before drinking and fermentation is not exercised. But, the present finding disagrees with the report of Noor *et al., (*2012) from the pastoral communities of Ethiopian Somali region who consumed camel milk in its raw and fermented state. This discrepancy is mainly due to the cultural diversity between these communities. Besides, the communities of the study areas do not have technical support and information access about the milk processing approaches.

According to the pastoralists view, milk from each species has its own unique attributes and properties. Pastoralists claim that camel milk is superior to the milk of other species. They gave many reasons for their preference of camel milk from milk of other domestic animals. Cows' milk tend to make people fat, that is, it causes obesity but camel milk gives strength, endurance and stamina, an attribute that pastoralists need in order to pursue a nomadic life style. Unlike cows' milk, camel milk has medicinal values and can be used to treat a number of aliments in human beings. This is similar to Mohammed *et al.,* (*2016*) respondents involved camel milk production in Afar region believe that camel milk had medicinal value when drank in raw state. In addition, 71.8% of producers or consumers are not aware about public health hazards of drinking raw milk.

Milk marketing is not practiced in the study area due to several factor including cultural believes, lack of information relating to milk marketing, remote lifestyle habits of camel rearing community, lack of technical support and infrastructure. The above factors embedded the marketing opportunities which can lead a better income to the camel rearing societies. Seifu, (2007) similarly reported in Ethiopia camel milk production and marketing activities in most pastoral areas experienced a lot of constraints mainly due to believes and lack of information of milk marketing. Odongo *et al.,* (2016) in Kenya also reported camel milk marketing is facing many constraints which are likely to lead to microbiological changes, resulting in high quality and quantity post-harvest losses.

The total bacterial count (TBC) is an indication of the sanitary conditions under which the milk is produced Ahmed *et al.,* (2010). Results obtained by enumeration of bacteriological content of raw camel milk samples of this study are shown in (Table 4). The TBC ranged from $2.3x10^9$ to $1.65x10^9$ cfu/ml with a mean value of $56.20x10^9$, $92.25x10^9$cfu/ml, being higher than those reported by Mulugojjam *et al.,* (2013). The total bacterial count results revealed that milk samples from buckets had significantly higher mean TBC ($P < 0.05$) as compared to that of milk samples directly taken from teat. The result of the present study indicated strong microbial contamination of the camel milk samples. And this was mainly due to poor sanitary conditions under and hands of milkers which the camel's milk was produced. This is similar with the report of Mulugojjam *et al.,* (2013). who reported an increase in ($p<0.05$) TBC was observed along the chain as the milk was transported from the production site until it reached the final markets in Harar and Dire Dawa towns in Ethiopia. And Abera *et al., (2016)* who reported that mean TBC showed a statistically significant ($P < 0.05$) increase from udder to market level. According to the Kenya quality standards for whole unpasteurized milk (KEBS, 2007), 51.6 % milk samples taken from Ab'ala exceeded the acceptable limits of 106 cfu/ml (grade III or fair) which indicates poor quality milk and a threat to human health.

No statistically significant variation was observed in CC in milk samples collected from the teat compared to milk samples from the equipment used to milk camels. Meanwhile, CC demonstrated a limited increase ($P > 0.05$) from udder (teat) to equipment (bucket) level (Table 5). The mean of milk samples taken from udder was $4.4x10^5$ cfu/ml and $6.147x10^5$ cfu/ml with the milk samples taken from the equipment used for milking of camels. Similarly, high coliform counts were observed in Moroccan camel milk (Benkerroum *et al.,* 2003). This is high coliform counts compared to the report for Afar by Semereab and Molla (2001) which were 3.472 x 10^3cfu/ml and 6.95 x 10^3cfu/ml for milk sampled from udder and milking bowl respectively. This is due to poor sanitary practices during milking and the handling practices observed during study.

Camel milk samples used in this study was found (52.5%) gram negative bacteria and (47.5) gram positive bacteria. Furthermore, *Staphylococcus* spp. (47.5 %), *E. coli* (32.5 %), and *Salmonella* spp. (20%) have been isolated from the raw camel milk samples (Fig 4). *Staphylococcus* spp. showed the highest prevalence at production level. Most of the bacterial genera identified are potentially pathogenic the risk of contamination of milk with pathogens is attributed to the practice of poor hygiene and handling practice of camel milk during milking and after milked. This is similar to the finding reported by Benkerroum *et al.,* (2004) who isolated bacterial pathogens including Streptococcus, Staphylococcus and Pseudomonas species from raw camel milk produced in Riyadh, Saudi Arabia.

Conclusion and Recommendations

The production system of the Afar region is dominated by pastoralism and extensive system in which camel household move depending on the water and pasture availability. Milking system was randomly and mixing the healthy and diseased animal which increase the incidence of disease. Majority of the respondents showed hand washing before milking is not applied in their camels. Generally the environment and milking

equipment used during the study time was unhygienic and similarly water was unclean. Camel milk is consumed in its raw state without boiling or processing which can lead threat to human health and therefore poses great health risk to the consumers. The handling system was poor due to unsystematic milking of she-camels, use of unclean utensils and storage of high temperature. Milk marketing is not practiced in the study area. Total bacterial count found in this study was high due to hygienic conditions. The total coliform count obtained in the present study was higher than acceptable limits. The presence of these coliform bacteria not only indicates the poor hygienic conditions in which milk was produced and unhygienic handling but also they could be pathogenic. The major isolates from milk samples were *Staphylococcus* aurous, *E. coli* and *Salmonella* spp. Therefore considering the above conclusions following recommendation should be implemented.

Strict hygienic control measures along milking and handling practice to improve hygienic conditions of milk from production to consumption and to enhance quality of milk.

Enhancement and promotions on the pastoralist's knowledge about the hygienic requirement to produce high quality milk.

Development of the knowledge of the pastoralist toward the milk-borne diseases.

Institutional support and awareness for improving of camel milk marketing.

Pathogenic bacteria which cause foodborne are seen in the study, so boiling of milk is mandatory.

Researchers and funding agencies should pay attentions to camels as they are the future livestock species in combating food security and environmental sustainability.

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Appendix.1 Questionnaire used for interviewing camel milk producers

Here by the following Questionnaire is set for the camel milk producers/consumers to carry out DVM thesis research on the title of, Assessment of bacteriological quality and handling practice of raw camel milk in ab'ala woreda of afar national regional state, Ethiopia**.** Background information concerning production systems milk production, handling and utilization practices at household level in ab'ala woreda was targeted. The personal profile obtained from the respondents with regard to the subject matter was kept confidential and will not have any consequence on the respondent in any way. Thus, please give your genuine response for contributions of your foot print to validate the information available at your area.

Your cooperation in completing the questionnaire is highly appreciated.

Name of the respondent: _____ Sex: _____

Kebele name: _____ Date: _____ Age: _____

Level of education

a) Illiterate b)Basic education c)Primary school d)Secondary school e)Technical education f)higher education

1. How many lactating camels do you have? ----------
2. How many liters of milk produced per lactating camel per day? ----------
3. Frequency of milking

A. Once a day B. Twice a day C. Three times a day D. Other--------

4. Do you clean the udder before milking? A. Yes B. No
5. Do you wash your hands before milking your she-camels A. Yes B. No
6. Do you clean the udder (teats) after milking? A. Yes B. No
7. Where do you usually milk the camel? A. In the open air B. Under the shade C. In the barn under the roof D. Other----------
8. What Type of material or vessels you use during milking?

A. Wood made B. Plastic made C. Clay made D. Fiber made E. Other

9. What types of materials (containers) commonly used for storage and processing of camel milk?

A. Wood made B. Plastic made C. Clay made D. Fiber made E. Other

10. Do you clean before and after milking the milk containers? A. Yes B. No
11. How do you clean the milk vessels?

A. By washing B. By smoking C. A and B D. Other

12. Source of the water used to clean the milk containers?

A. Tap water B. Well water C. Spring water D. streams E. Other----

13. Do you boil the water before you use it to clean the vessels? A. Yes B. No
14. Why do you milk the came for what purpose?

A. Home consumption B. Sale C. Both D. Other----------

15. In what form do you consume camel milk?

A. Raw milk B. fermented C. A&B D. mixed with other milk

D. Other----

16. Do you undertake processing of camel milk? A. yes B. No
17. Which processed product of camel milk is commonly used in your area?

A. fermented B. butter C. cheese D. A & B E. Other...

18. If your choice is raw camel milk, why you prefer it?
19. How do you handle the containers used for milking and storage of milk?
20. What are the procedures of milking of she-camel?
21. How do you keep the fresh milk until it is sold or consumed?

A. In a relatively cool place B. In a hot place C. Keep it at room temperature

D. Where ever no problem E. Other

22. Does fresh milk get spoiled? A. Yes B. No
23. If yes, mention types and causes of spoilage?
24. The milk of which animal do you mostly prefer to drink?

A. Camel B. Cattle C. Goat D. Sheep
26. Does camel milk have medicinal value?
A. Yes B. No
If yes, what are the diseases cured using it and the amount of milk used
27. Personal hygiene of the milkers and the preconditions they use during milking
28. Do you believe that the raw milk you produce is safe for consumption? Yes_____ No
If your answer is no, describe
29. Do you sell your camel milk? a. Yes b. No
If yes, how many litters per day? ----------, If no why?
30. Do you have access to veterinary service to the lactating camels?
a. Yes b. No
31. Do you have information access about milk related health problems?
32. Do you know that milk can induce disease to the consumers? a. Yes b. No
33. What are the common diseases in the milking camels? ----------
34. What are the commonly used drugs to treat your milking camel? ----------
35. Do you know about withdrawal period? a. Yes b. No
If yes, what is the importance of withdrawal period? ----------
36. Do you follow withdrawal period? a. Yes b. No
37. Do you milk diseased she-camels? a. Yes b. No
If yes, which one do you milk first? Diseased or healthy?

Appendix.2 Data pictures in the laboratory and in the field