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Microbiological Quality of Raw Camel Milk across the Kenyan Market Chain

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ABSTRACT

The objective of this study was to determine the microbiological quality of camel milk at critical points along the market chain. 36 camel milk samples were assessed by plating counts of total bacteria (TBC), *Streptococcus/Enterococcus* (PSEC), yeast and mold (YMC), *Enterobacteriaceae* (EBC), and *Staphylococcus* (PSC). At milking level all milk samples had TBCs not exceeding 10^5 cfu ml^{-1}. EBC exceeding 10^3 cfu ml^{-1} indicating grade II quality was found in 25% of primary collectors' milk. 75% of bulked milk at the final market had TBC exceeding 10^6 cfu ml^{-1} and EBC of 5.0×10^4 cfu ml^{-1}; grade III and IV quality of raw milk an indicator of poor quality and threat to human health according to Kenya quality standards. All microbiological counts increased along the market chain with milk pH changing from 6.49 at milking level to 6.39 at final market. The air and water at the milking level were grossly contaminated while milk containers at milking and primary collection centers needed more appropriate sanitization procedures.

Keywords: camel milk quality, camel milk safety, pastoralism
Abbreviations: ASAL, arid and semi-arid lands; **cfu ml^{-1}**, colony forming units per milliliter; **EBC**, *Enterobacteriaceae* count; **PSC**, presumptive, *Staphylococcus* count; **PSEC**, presumptive *Streptococcus/Enterococcus* count; **TBC**, total bacteria count; **YMC**, yeast and mold count

INTRODUCTION

Camel husbandry in Kenya is mainly conducted in the arid and semi-arid land (ASAL) regions with daily milk yields of between 3 to 10 kg of milk per camel in a lactation period of 12 to 18 months (Farah *et al.* 2007). However, there are several constraints in camel milk production and marketing; clean water for washing containers is scarce or unavailable, common use of recycled oil plastic jerry cans with small opening and long durations during transportation in high ambient temperatures, among other factors (Younan and Abdurahman 2004). Thereby microbial spoilage of camel milk inevitably reduces market value and freshness of marketed milk reducing the income to producers and vendors.

According to De-Buyser *et al*. (2001), Leclerc *et al*. (2002) and Harrington *et al*. (2002) public health concern associated with microbial food safety has arisen with certain *Enterobacteriaceae* (e.g. *Escherichia coli*, *Salmonella* spp., *Shigella* spp.), *Staphylococcus aureus*, pyogenic streptococci, *Campylobacter jejuni*, *Listeria monocytogenes*, some *Brucella* spp., *Yersinia enterocolitica* and pathogenic molds. Milk has been identified as a vehicle of these organisms in many occasions (Harrington *et al*. 2002). For instance, mastitis in camels has been studied in the regions bordering Kenya: Ethiopia (Abera *et al*. 2010), Somalia (Abdurahman 2006) and Sudan (Obied *et al*. 1996). In addition, coagulase-negative/ coagulase-positive *Staphylococcus aureus*, *Streptococcus agalactee*, Shigatoxigenic *E. coli*, *Bacillus* spp., and pathogenic molds have been isolated from camel milk in Kenya, Ethiopia and Sudan (Abera *et al.* 2010; Ahmed *et al.* 2010; Njage 2010). Poor hygiene is a major cause of spoilage of milk products (Brokken 1992; Farah 2004) with zoonotic and human fecal microorganisms posing an important public health threat to consumers with traditional preference for raw camel milk (Younan and Abdurahman 2004). Currently, the main focus in Kenya is on improving the camel milk microbiological quality and safety. Proposed interventions have included the use of lactoperoxidase system activated with commercial LP-system kits on pooled camel milk at collection centers (Njage and Wangoh 2008), introduction of cooling facilities, pasteurization of milk, and provision of clean water and training on hygiene handling of milk (Younan and Abdurahman 2004; Farah *et al*. 2007; Musinga *et al*. 2008; Kaindi 2009; Kamau *et al*. 2010; Njage 2010).

Therefore it was necessary to investigate the microbiological contamination of camel milk along the informal market chain by assessing safety and quality indicator organisms to obtain baseline situation of marketed camel milk. The contamination of air at milking area, water for sanitizing milk containers, and containers at milking level/primary collection point were also evaluated.

MATERIALS AND METHODS

The study was carried out in Nanyuki and Isiolo Counties in Kenya. Nanyuki is located at latitude 0° 1′ 0″ North and Longitude 37° 4′ 0″ East while Isiolo is at latitude 0° 21′ 0″ North and longitude 37° 35′ 0″ East. In each region, three camel herds were selected in order to capture two herd management practices (semi-modern ranching and pastoral/ traditional systems) which had well defined market chains.

Sample collection

The sampling procedure of Bonfoh *et al*. (2003) was used with the milking level, primary collection point in the local center (Nanyuki/Isiolo) and final market in Nairobi identified as the critical points along the market chain. Samples at milking level were col-

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Table 1 Growth media, preparation and incubation conditions of micro organisms of interest.

Growth media	Media preparation	Incubation conditions	Supplements	Cultivated organisms of interest	Reference strains
KF Streptococci Agar (Difco)	Boiling for 1 min in a water bath	43°C for 24-48 h, aerobic	1% 2,3,5-triphenyl tetrazolium chloride solution (Merck)	Enterococci/ presumptive streptococci (PSEC)	*Enterococci faecalis* JH-2-2
Baird Parker Agar (Biolife)	Autoclave at 121°C for 15 min	37°C for 24 h	5% Egg yolk tellurite emulsion (Biolife)	Presumptive staphylococci (PSC)	*Staphylococcus aureus* RN4220/PVC5
Yeast Mould Agar (Difco)	Autoclave at 121°C for 15 min	30°C for 2-3 days	Chloramphenicol at 20 mg/L (Fluka)	Yeast and molds (YMC)	*Rhodotorula mucilaginoasa* FSQE63
Violet Red Bile Glucose Agar (Merck)	Boiling for 1 min in a water bath	37°C for 24 h	-	*Enterobacteriaceae* (EBC)	*Escherichia coli* Xl1-Blue
Plate Count Agar (Difco)	Autoclave at 121°C for 15 min	30°C for 24 h	-	Aerobic mesophilic bacteria (TBC)	-
MacConkey broth	MPN method	-	-	Coliforms in washing water	-

Table 2 pH, temperature of camel milk at different points along the market chain, time elapsed between critical points and the prevailing environmental temperature.

Sample description	n*	pH*	Milk temperature* (°C)	Environmental temperature* (°C)	Cumulative time (hours) elapsed
Individual animal milk	11	6.49 ± 0.1	26 ± 2.3	23.5 ± 6.6	< 0.5
Bulked morning milk at herd level	5	6.49 ± 0.1	28 ± 0.8	24.5 ± 6.0	1
Bulked milk at 1st collection point	5	6.46 ± 0.1	29 ± 1.7	25 ± 4.6	4 - 8
Bulked milk at final market (Nairobi)	5	6.39 ± 0.1	10.5 ± 0.5	20	21-25

n* = number of samples; pH* = mean pH ± confidence interval (CI); Milk temperature* = Mean ± CI; Environmental temperature* = Mean ± CI

lected between 6.00-7.00 am. Milk from 18 lactating camels was obtained singly into 50-ml sterile Falcon tubes after milkers' hands and camel udder had been disinfected with 70% ethanol using a hand sprayer and dried with a disposable towel. Six samples were collected from bulked camel milk at milking level while same number of samples was collected at primary collection point and final market from milk containers followed up along the market chains. Upon milk sample collection pH of all milk samples was measured using a digital pH-meter (High-precision 780 pH Meter, Metrohm AG, Switzerland). Up on sample collection, milk and prevailing environment temperature were measured and recorded in a field questionnaire. The milking yard was assessed for environmental TBC and YMC by exposing two Petri dishes containing either Standard Plate Count Agar (Difco, USA) or Yeast Mold agar (Difco, USA). Contamination of milk containers at the milking level and primary collection point was determined using the rinsing technique with 100 ml of sterile water. A sample of water (100 ml) used for cleaning containers at the milking level and primary collection point were collected into sterile tubes. California Mastitis Test (CMT- test) was carried out on milk obtained from each individual animal before bulking. Labeled samples were then transported for analysis within 12 h to a laboratory in Nairobi in a cool box containing adequate dry ice.

Microbiological analysis

Serial dilutions of samples were prepared using sterile dilution solution of 0.85% of sodium chloride (NaCl) and 0.1% of peptone from casein. Appropriate triple series dilutions were prepared and 0.1 ml surface-plated in duplicate onto appropriate selective or semi-selective growth medium for enumeration of specific groups of microorganisms (**Table 1**).

Statistical data analysis

All data obtained in the field questionnaire and from bacteriological analysis was entered in Microsoft Access database. Statistical data analysis was carried out using Intercooled Stata Version 9.0 (Stata Corp., College Station, TX, USA, 1984–2000). Data on the microbial counts was first transformed to logarithm of colony forming units per milliliter of sample (log cfu ml^{-1}) and the results were presented as the geometric means and other descriptive statistics. Linear contrast was carried out to compare the counts at different sampling points along the market chain while one-way analysis of variance was used to compare data from the two herding systems.

RESULTS

pH, temperature of camel milk, environmental temperature and time elapsed between identified critical points along the market chain

There was a slight decrease in the pH of camel milk at critical points along the market chain (**Table 2**). The pH of bulked milk at the herd level was 6.49 and decreased to 6.39 at the final market in Nairobi. The milk temperature at milking during the cold and warm weather was between 27-29°C (**Table 2**) with environmental temperature of 17-21°C. The temperature of the milk on arrival at the primary collectors was about 29-30°C with environmental temperature of 24-30°C. At the final market the temperature of milk was between 10-11°C as a result of refrigeration at the primary collection point before milk transportation to final market in Nairobi. The time elapsed between milking and primary collection point was 2.75-6.5 h while the milk took 18.75-24.75 h between the primary collection point and final market in Nairobi.

Contamination of milking area air, washing water, containers at the milking level and at primary collection point

Microbial quality of milk containers at the milking level and primary collection point was not significantly different ($P > 0.05$) (**Fig. 1**). The containers at the milking level had the following counts (cfu ml^{-1}), TBC 10^1-10^5, PSEC 10^3, EBC 10^4, YMC 10^2-10^3 and PSC 10^2-10^3 while containers at primary collection point had TBC 10^2-10^5, PSEC 10^2-10^4, EBC 10^1-10^5, YM 10^2-10^5 and PSC 10^1-10^4. Water at the herd level was heavily contaminated with more than 1.8×10^2 coliforms per milliliter of water and TBC ranging from 10^3-10^5 cfu ml^{-1}. The air at the milking yard had TBC 10^2-10^3 and YMC 10^2 cfu per plate.

The microbiological quality of camel milk

The prevalence of mastitis among lactating individual camels was 29% (5/17) at the milking level. The geometric means of the microbial counts and the range of counts between the main points along the market chain are shown in **Table 3**. Pair-wise comparisons of all the organisms with exception of PSEC in milk obtained from individual animals before and after pooling at the milking level were not

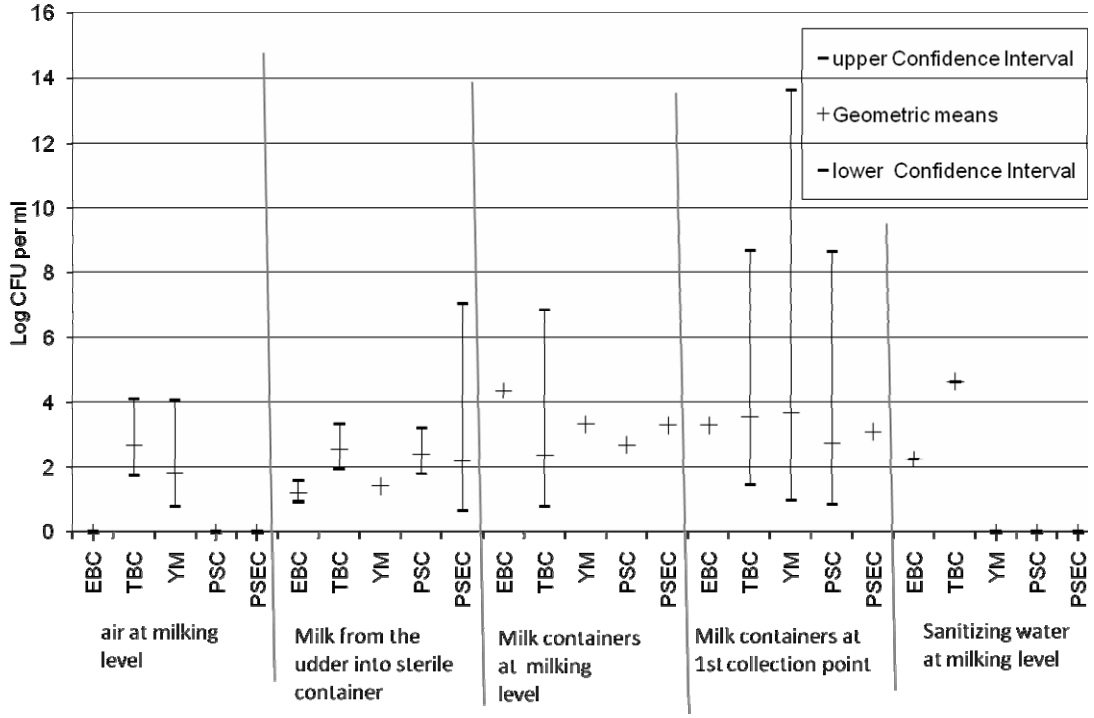

Fig. 1 ***Enterobacteriaceae*** **(EBC), Total bacterial counts (TBC), yeast and mold (YMC), counts for presumptive** ***Streptococcus/ enterococcus*** **(PSEC), and presumptive** ***Staphylococcus aureus*** **(PSC) in (air, aseptically obtained milk, water, containers) at milking level and containers at 1st collection point of informally marketed camel milk in Kenya.**

Table 3 Total bacterial counts (TBC), counts for presumptive *Streptococci/ enterococci* (PSEC), yeast and mold (YMC), *Enterobacteriaceae* (EBC)and presumptive *Staphylococci* (PSC) in raw camel milk along the market chain in Kenya.

Presumptive microorganisms	Milk from udder (aseptic conditions) (*n=18)		Pooled milk at milking (n=6)		Primary collectors (n=6)		Final market (Nairobi) (n=6)	
	Geometric mean (cfu ml^{-1})	Range of counts (cfu ml^{-1})	Geometric mean (cfuml^{-1})	Range of counts (cfu ml^{-1})	Geometric mean (cfu ml^{-1})	Range of counts (cfu ml^{-1})	Geometric mean (cfu ml^{-1})	Range of counts (cfu ml^{-1})
TBC	3.6×10^2	2.1×10^1-4.7×10^4	3.2×10^3	9.2×10^2-1.7×10^4	5.9×10^4	1.1×10^3-5.6×10^5	3.2×10^6	4.7×10^5-1.0×10^7
PSEC	1.7×10^2	2.1×10^1-1.4×10^3	7.1×10^1	3.7×10^1-3.4×10^2	3.9×10^2	3.1×10^1-2.7×10^4	4.4×10^3	2.0×10^2-5.4×10^4
YMC	2.8×10^1	1.1×10^1-1.0×10^2	6.2×10^1	2.1×10^1-2.7×10^2	1.2×10^2	1.1×10^1-5.0×10^4	1.4×10^3	9.8×10^2-3.2×10^3
EBC	1.8×10^1	1.1×10^1-8.1×10^2	5.2×10^1	1.1×10^1-8.1×10^2	9.5×10^1	1.0×10^1-3.0×10^5	1.6×10^5	1.4×10^4-3.5×10^6
PSC	2.4×10^2	1.8×10^1-2.4×10^4	1.3×10^3	3.5×10^2-8.3×10^3	6.3×10^3	6.0×10^2-8.2×10^4	2.0×10^5	9.1×10^4 -2.8×10^5

* n = number of samples

statistically different. Bacterial counts correlated significantly ($P > 0.05$) to time taken and point along the market chain except for YMC. Bacterial counts in milk from semi-modern ranching and traditional camel husbandry were not statistically different.

DISCUSSION

pH, temperature of camel milk, temperature of the environment and time elapsed between points along the market chain

Raw bulked milk microbial quality depends on among other factors temperature at which milk is stored and time elapsed between milking and collection (Soler *et al*. 1995; Aumaitre 1999; Ahmed *et al*. 2010). Camel milk was observed to take more than 6 hrs without cooling before it arrived at primary collection point and 21-25 h between the latter and final market in Nairobi. Owing to the high ambient temperatures of up to 30°C and lack of cooling system, milk reaches the primary collectors at elevated temperatures of up to 30°C. Milk reached the final market in Nairobi at 10-11°C but, occasionally it may be higher due to delays during transportation by bus (pers. obs.). Long delays in camel milk delivery observed in this study, could be explained by delays during transportation as a result of poor infrastructure or long distances between production areas and final market (Farah *et al.* 2007).

The pH of camel milk was 6.3-6.5 similar to findings from Farah (2004) and Ahmed *et al*. (2010). The stability of camel milk pH due to its buffering phenomenon as described by Attia *et al*. (2001) was also observed in this study since the pH at final market was acceptable at 6.39 even though total titratable acidity indicated that the milk was already souring (personal observation). It is difficult to observe visual changes due to souring since camel milk does not form a firm coagulum (Yagil *et al*. 1983; Wangoh 1997; Attia *et al*. 2001; Younan and Abdurahman 2004; Kamau *et al*. 2010).

Camel milk contamination factors

The contamination factors along the production and informal market chain reduce the shelf life, quality and safety of camel milk. The air at the milking area had high TBC and YMC showing possibility of milk contamination during milking and/ or storage if milk containers were left open. The milking area was also dusty and hence possibility of contamination from microorganisms from soil, from milkers' hands or camel coat during milking (Younan and Abdurahman 2004; Musinga *et al*. 2008).

Sanitation and water hygiene are extremely important if contamination of milk is to be avoided (Gran *et al*. 2002). In this study, water at the milking level was sourced from rivers or lagoons with coliforms counts more than 180 cfu ml^{-1} and high TBC making it an important source of milk contamination if the water is not adequately heated before washing of containers. Water for cleaning milk containers should be clean potable water (Lore *et al*. 2006; Musinga *et al*. 2008). Farah (2004) noted that water in the ASALs is grossly contaminated and its availability in the camel milk production areas is scarce or unavailable, thus making it difficult to improve milk hygiene at the milking level. The main source of water at the primary collection point was potable water from a borehole. Majority of milk containers were cleaned at the primary collection point rather than at the milking level. However, after using treated municipal water and container smoking, containers was at the primary collection point was not significantly different from containers at the herd level. This shows that appropriate container sanitizing procedures are not adhered to.

Containers for milking, transportation, and storage of milk should be adequately cleaned/ disinfected to avoid microbial contamination (Lore *et al*. 2006). In this study, the commonly used containers for handling, storage, and transportation of camel milk were plastic jerry cans of varying sizes. Since, many containers used are of small capacity and have a small opening which creates difficulty during cleaning (Bonfoh *et al*. 2003; Wangoh 2004; Bonfoh *et al*. 2006; Ahmed *et al*. 2010). Even though, the use of detergents and good quality water during cleaning of equipments improves the microbiological quality of milk (Bonfoh *et al*. 2006; Musinga *et al*. 2008). Cleaning of containers and disinfection with either chemicals or hot water was not a common practice.

The prevalence of mastitis of individual animal milk before bulking at milking level was 29% similar to that reported by Younan and Abdurahman (2004) and Abera *et al*. (2010) in Ethiopia. Presence of food-borne pathogens in bulked milk can also be linked to feacal contamination during milking, or from lactating camels with mastitis (Younan *et al*. 2001; Younan and Abdurahman 2004; Oliver *et al*. 2005; Ahmed *et al*. 2010; Obied *et al*. 2010; Megersa *et al*. 2011; Tesfaye *et al*. 2011).

It is therefore clear that, many interactive factors contributed to poor hygienic quality of the camel milk sold at the markets. Younan and Abdurahman (2004) reported several risk factors; little consideration to hygiene, pooling of morning and evening milk at milking level and bulking milk from different camel herds and intense manipulation of small quantities of milk using several containers of small capacity at the primary collectors, transportation and handling without any cooling were also observed during this study.

Milk microbiological quality

The Kenya Bureau of standards (KEBS) (2007) on raw whole camel milk specifications was used in this study. TBC in camel milk milked directly into a sterile conical flask was 10^2-10^4 cfu ml^{-1} similar to results reported by Younan and Abdurahman (2004) and Farah *et al*. (2007). However, their findings of bulked milk at milking level was10^3-10^5 cfu ml^{-1}, primary collection point at 10^6-10^7 cfu ml^{-1} and final market at 10^6-10^8 cfu ml^{-1}, were higher than the findings of this study. The current findings were also in agreement with those of camel milk in Qassim region which had mean counts of 10^5 cfu ml^{-1} and maximum of 10^7 cfu ml^{-1} (El-Ziney and Al-Turki 2007), mean counts of 10^5 cfu ml^{-1} in Saudi Arabia camel milk (Al Mohizea 1994) and 10^6 cfu ml^{-1} in Ethiopia (Semereab and Molla 2001).

75% of the bulked milk at primary collection point was within the microbiological acceptable limit of 10^6 cfu ml^{-1} indicating milk of grade I and II quality while 75% bulked milk at the final market exceeded the microbiological acceptable limits of 10^6 cfu ml^{-1} (grade III and IV) of raw milk (KEBS 2007) which indicates poor quality milk and a threat to human health. There was significant increase or buildup of TBC in milk between the primary collection point and the final market.

Presumptive *Streptococcal/Enterococcal* counts were significantly different between the primary collection point and final market in Nairobi which had mean 10^3 cfu ml^{-1} and a maximum count of 10^4 cfu ml^{-1}, probably as a result of microbial build up due to long storage period of market milk and further contamination at the primary collection point.

At primary collection point , yeast and mold count in this study had maximum counts of 10^4 cfu ml^{-1} which was slightly lower than mean and maximum values of 10^2 and 10^6 cfu ml^{-1}, respectively for the Qassim region (El-Ziney and Al-Turki 2007), and Moroccan camel's milk with mean count of 10^6 cfu ml^{-1} (Benkerroum *et al*. 2003). According to Frazier and Westhoff (1998) and Pitt and Hocking (1997), the high YMC in milk are uncommon since the the natural pH of milk cause bacteria to predominate. However, the FAO (1992) reported that, yeast and molds are able to grow in a wide pH of 2-9 and in many cases they alter the pH of milk to about 4-6.5 favourable to their growth.

The EBCs increased from 9.5×10^1 to 1.6×10^5 cfu ml^{-1} between primary collection centers and final market indicating significant contamination and microbial build up at this point. Twenty five percent of bulked camel milk at primary collection point had EBC exceeding 10^3 cfu ml^{-1} indicating grade II quality of milk while 75% of bulked final market milk had EBC exceeding microbiological acceptable limit of 5×10^4 $cfuml^{-1}$ indicating milk of grade III quality (KEBS 2007). The results are in agreement with the findings of camel milk in Qassim region with a mean value of 10^3 cfu ml^{-1} and a maximum of 10^7 cfu ml^{-1} (El-Ziney and Al-Turki 2007). Similarly, high coliform counts were observed in camel milk in Ethiopia (Semereab and Molla 2001) and in Moroccan camel milk (Benkerroum *et al*. 2003) which was 10^7 cfu ml^{-1} on average. However, existence of coliforms may not necessarily indicate a direct fecal contamination of milk, but is an indicator of poor sanitary practices during milking and further handling processes (Frazier and Westhoff 1988).

The PSC counts of bulked camel milk at the farm and primary collection point had mean counts of 10^3 cfu ml^{-1} while at final market it was 10^5 cfu ml^{-1}. The mean counts of PSC in bulked milk are in agreement with findings of Moroccan camel milk 10^5 cfu ml^{-1} and were slightly lower than camel milk in the Qassim region in Saudi Arabia with mean count of 10^7 cfu ml^{-1} (El-Ziney and Al-Turki 2007).

In this study, camel milk quality control checks are unavailable, with most buyers and sellers relying on organoleptic testing, hence, milk microbiological quality deteriorates unnoticeably. Milk rejects have discouraged many camel farmers from supplying the Nanyuki camel milk dairy which has quality control measures in place. Majority of pastoralists believe that camel milk has unique beneficial properties which are lost by heating close to boiling temperature and thereby preferred consumption of raw or unpasteurized camel milk for its medicinal or therapeutic purposes. We therefore underline that consumption of unprocessed camel milk at the current status poses potential public health risk as was reported in other studies (Kaufmann and Binder 2002; Younan and Abdurahman 2004; Farah *et al*. 2007; Njage 2010).

CONCLUSION AND RECOMMENDATIONS

The microbial quality of camel milk was the same for semi-modern ranching and traditional camel husbandry. Milk at the milking level had TBCs not exceeding microbiological limit of 10^5 cfu ml^{-1} and was ranked a grade I quality milk. At primary collectors 25% had EBC exceeding 10^3 cfu ml^{-1} indicating grade II quality of milk while 75% was grade I quality. However, 75% of bulked milk at the final market exceeded the TBC acceptable limits of 10^6 cfu ml^{-1} and EBC of 5.0×10^4 cfu ml^{-1} (grade III and IV quality of raw milk) according to KEBS (2007) an indicator of poor quality and a threat to human health.

Therefore, in order to safeguard consumer health and to strengthen the source of income through the sale of milk by producers and vendors, there should be initiatives to lower microbiological contamination of camel milk at milking level, primary collectors at the local centers and final market. Training on hygiene handling of milk for herders, the primary collectors and vendors is also necessary. Other interventions should focus on provision of clean water at milking level, veterinary services, reduce the time taken before selling milk, provision of milk cooling facilities at milking level and other levels and provision of efficient/ organized milk transportation and storage systems.

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