

**ADDIS ABABA UNIVERSITY**  
**COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**ASSESSMENT OF BACTERIOLOGICAL QUALITY OF CAMEL RAW MILK IN  
SELECTED PASTORIAL KEBELES, FENTALE DISTRICT, OROMIYA REGION,  
ETHIOPIA**

**BY**

**AKLILU FELEKE HAILE**

**JUNE 2012**

**DEBRE ZEIT, ETHIOPIA**

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**AKLILU FELEKE HAILE**

**A thesis submitted to the School of Graduate Studies of Addis Ababa University in  
partial fulfillment of the requirements for the Degree of Master of Veterinary Science in  
Tropical Veterinary Public Health**

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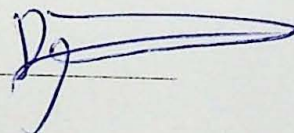
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## LIST OF ABBREVIATIONS

|         |                                              |
|---------|----------------------------------------------|
| CFU/ml  | Colony Forming Unit per Milliliter           |
| CNS     | Coagulase Negative <i>Staphylococcus</i>     |
| CPS     | Coagulase Positive <i>Staphylococcus</i>     |
| FAO     | Food and Agriculture Organisation            |
| FAOSTAT | Food and Agriculture Organization Statistics |
| O-F     | Oxidation Fermentation                       |
| NAGase  | N-Acetyl-Glucosaminidase                     |
| Q fever | Query fever                                  |
| SPC     | Standard Plate Count                         |
| TBC     | Total Bacterial Count                        |
| TCC     | Total Coli form Count                        |
| TSC     | Total Staphylococcus Count                   |
| UAE     | United Arab Emirates                         |
| USA     | United States of America                     |
| USSR    | Union of Soviet Socialist Republics          |
| VTEC    | Verotoxigenic <i>Escherchhia Colli</i>       |
| WHO     | World Health Organization                    |



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## ABSTRACT

The current study was undertaken to assess the bacteriological quality of raw camel milk and to isolate and identify prevalent bacterial species from raw camel milk from study areas. A total of 420 milk samples (384 from lactating camels and 36 from milking utensils) were collected from August 2011 to April 2012. An observational study was conducted to gain an insight on pre-harvest, harvest and post-harvest milk hygiene practices. The standard plate count and coliform count as well as isolation and identification of bacteria from camel milk were carried out by employing standard microbiological techniques. The mean standard plate count for milk sample taken from the udder of lactating camels and milking utensils were  $4.30 \log_{10}$  CFU/ml and  $5.34 \log_{10}$  CFU/ml, respectively. A statistically significance difference was observed between the two means ( $P= 0.000$ ). The mean coliform count of milk samples collected from the udder of lactating camels and milking utensils were  $0.98 \log_{10}$  CFU/ml and  $3.57 \log_{10}$  CFU/ml, respectively. There was also a statistically significant difference between the two mean ( $P= 0.000$ ). Clinically mastitic udder have role as a source of *Staphylococcus aureus*, *S. hyicu*, *Streptococcus agalactiae*, *S. dysgalactia*, *Enterococcus faecalis*, *Bacillus* spp., *Escherichia coli* and *Mannheimia hemolytica* in camel milk. Subclinical mastitis acts as a source of contamination for microbes such as *Staphylococcus aureus*, *S. hyicus*, *S. intermedius*, *Enterobacter aerogenes*, *Escherichia coli* and *Corynebacterium* spp..Likewise, milk form the also yielded several bacteria which included *S. aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. uberis*, *Enterococcus faecalis*, *Klebsiella pneumonia* and *Pseudomonas* spp.. On the other hand, milking utensils served as an important source of *Escherichia coli* and *Staphylococcus epidermidis*. A higher bacterial counts was observed in milk samples taken from milking utensils as compared to the milk samples collected directly from the udder indicating poor milk hygiene/ manufacturing practices. The prevalence of infectious mastitis can result in reduced milk yield and poor quality of milk. In addition, consumption of raw camel milk by herdsman may pose considerable health hazards.

**Key words:** Bacterial load, Camel, Mastitis, Raw milk



## 1. INTRODUCTION

Camels were first domesticated 3,000 years ago (Epstein, 1971). Countries in the Horn of Africa i.e. Ethiopia, Somalia, Sudan, Kenya, Djibouti and Eritrea have proportionately higher number of camels (one humped) than any other part in the world (FAOSTAT database, 2005). Camels are important milk producers in arid lands, and camel milk is an essential food for livelihood of people and it may be the only milk available in places where other milking animals cannot be maintained. According to FAO's report, more than 18 million camels around the world support the survival of millions of people (FAO, 2003). Camel milk not only contains comparable nutrients compared to cow milk, but also it has therapeutic and antimicrobial agents (Konuspayeva *et al.*, 2009). It is popular among pastoralists and has been steadily gaining popularity among urban dwellers in many countries. In Ethiopia, the annual camel milk production was estimated to be 75, 000 tones (Feleke, 2003). The entire camel population of the country is maintained in the low input production system of the lowland areas and all camels are of indigenous breeds.

Camels are considered as resistant to many diseases. Currently, camel mastitis is being reported with higher number of prevalence from several camel rearing countries. The pathogenic bacteria isolated are similar to those reported from other milk animals such as cow, buffalies, goats and sheep. The clinical and subclinical mastitis are reported from Ethiopia with higher prevalence, up to 12% and 46.2%, respectively (Abdel Gadir, 2001; Bekele and Molla, 2001).

Raw milk which is aseptically drawn from a healthy animal usually contains few bacteria, and the first contamination of milk may occur at the moment of milking during the passage of the teat canal and by milking equipment or milking personnel (Pal, 2012). Further contamination takes place during transport and storage of the milk. In addition to mastitis, poor management and unhygienic milking practices prevalent in the traditional husbandry systems are few of the factors responsible for contamination of milk (Almaw and Molla, 2000; Pal, 2012).



The hygienic status of raw camel milk is influenced by the microbes that is present in it. There are some investigations conducted to assess the hygienic status and bacterial load of raw camel milk in different camel rearing countries. This is indicated by the general indicators, like the total bacterial count (TBC) and the total coliform count (TCC), and the report of specific pathogenic bacteria. The reported TBCs in camel milk vary between  $10^2$  and  $10^8$  cfu/ml. The TCCs are also reported with higher values and the prevalence ranged from 1.4 % up to 29.4 %. Specific organisms which are given emphasis and reported from raw camel milk include *Staphylococcus* spp., *Salmonella*, *Bacillus cereus*, *Streptococcus* spp., *Listeria* spp., *Mannheimia haemolytica*, *Pseudomonas aeruginosa*, *Micrococcus* spp., *Arcanobacterium pyogenes* and other anaerobic bacteria like; *Clostridium perfringens*, *Fusobacterium necrophorum*.

Investigations on the bacteriological quality/load of camel milk revealed that most of the milk samples collected from milking vessels contained higher number of bacteria and they have also additional type of bacteria like *Pseudomonas* spp. and *Bacillus* spp. (Hadush *et al.*, 2008). These authors reported that majority (68.59%) of milk samples from milking bowls were having grades of fair, poor or very poor while 94.12 % of the udder milk samples were having very good grade. Raw camel milk contamination by *Salmonella enterica* is influenced by post-harvest handling of the product rather than camel infection by the pathogen (Matofari *et al.*, 2007). The absence of proper transportation and cooling chains often results in uncontrolled fermentation and growth of unwanted microorganism in milk.

Fentale District is one of the camels rearing areas in Ethiopia. Though some studies are conducted in different parts of Ethiopia on the prevalence of camel mastitis and in some cases on the bacterial load of raw camel milk, there is no similar work in Fentale District. Moreover, 11 of the 18 Peasant associations in pastoral area, are accustomed to consume raw milk. It was, therefore, necessary to know the present hygienic situation on the raw camel milk by conducting detailed microbiological examination of raw milk. Therefore, the present study was contemplated with the following objectives:

- To assess the bacterial load of raw camel milk collected from dairy camels and milking utensils.



- To isolate and identify the types of bacteria spp. present in raw camel milk and milking utensils.
- To observe camel milk hygienic practices in the study area.

## 2. LITERATURE REVIEW

### 2.1. Camel milk

#### 2.1.1. Camel lactation and camel milk yield

Camels can lactate under severe drought conditions even when other milk-bearing animals perish. The quality of the milk under drought conditions is also admirably suited to human requirements. The length of the lactation period depends on breed, parturition, climatic and feed conditions and is reported to average between 12 months (Farah, 2004) to 18 months (Rao *et al.*, 1970). During the first 3 months of lactation, their milk yield increases significantly. After a peak during the fourth to fifth month, it starts to decrease (Simpkin *et al.*, 1997; Gaili *et al.*, 2000; Wernery *et al.*, 2004).

East Africa harbors an estimated 11 million camels (58% of the world camel population) which produce over two third of the world's annual camel milk volume of 1.3 million tones. In Kenya, camel milk constitutes 12% of 3 billion liters of the milk produced nationally and the average camel milk production per annum is 366,000 liters and 25% of the national population depends on camel milk (Matofari *et al.*, 2007). In Ethiopia, the annual camel milk production was estimated to be 75, 000 tones (Feleke, 2003). The entire camel population of the country is maintained in the low input production system of the lowland areas and all camels are indigenous breeds.

The milk yield varies between the different dromedary breeds or types and between individual camels of the same breed. High milking frequency and good, adequate feed have also a positive effect on milk yield. Some dairy breeds are characterized by a milk production capability of more than 2,090 kg up to 4,000 kg per lactation (305 d) under natural grazing conditions (Simpkin *et al.*, 1997). An overview on the reported milk yields is given in Table 1. The average daily yield lies between 4.0l in UAE (Wernery *et al.*, 2006) and a maximum value of 18.7 l in Pakistan



(Knoess *et al.*, 1986). The usual range of daily yield is given with 2.4 – 7.6 l. There is also a great variety in the reported average lactation yield: 1,422 kg – 5,695 kg.

Table 1: Milk yield in camels reported from various sources

| Author(s)                      | Country         | Yield<br>kg/d | Yield range<br>(kg/d) | Average<br>yield <sup>1</sup><br>(Kg/lact.) | Yield range <sup>1</sup><br>(Kg/lact.) |
|--------------------------------|-----------------|---------------|-----------------------|---------------------------------------------|----------------------------------------|
| Knoess <i>et al.</i> , 1986    | Pakistan        | 18.7          | -                     | 5,695                                       | -                                      |
| Basmaeil and Bakkar, 1987      | Saudi<br>Arabia | 5.5           | 2.4 -7.6              | -                                           | -                                      |
| Simpkin <i>et al.</i> , 1997   | Kenya           | -             | 5.7 - 6.6             | 2,670                                       | 1,386-4,146                            |
| Gaili <i>et al.</i> , 2000     | Pakistan        | -             | -                     | -                                           | 1,048-2,576                            |
| Wernery <i>et al.</i> , 2004   | UAE             | 5.0           | 3.0 - 7.0             | -                                           | -                                      |
| Khaskheli <i>et al.</i> , 2005 | Saudi Arabia    | -             | 3.5 - 35.0            | -                                           | 4,575-20,675                           |
| Wernery <i>et al.</i> , 2006   | UAE             | 4.0           | 3.1 - 7.5             | -                                           | -                                      |

<sup>1</sup>In most articles, the average lactation period mentioned was 305 days.

## 2.1.2. Composition and characteristics of camel milk

### *Camel milk composition*

Camel milk has a white opaque colour, a faintly sweetish odour and a sweet but sharp taste. It is thinner than cow or buffalo milk. Camel milk has a much slower natural creaming rate than cow milk (Farah, 1993). The moisture content is 86.0 - 90.5 %, which can be compared to that of cow, goat or human milk. The water content in camel milk increases during lactation and with parities (age) and under conditions of dehydration (Farah, 2004; Gaili *et al.*, 2000). Fat content is reported with values between 2.0 and 4.2 % (Alshaik and Salah, 1994). The fat content decreases with the progress of lactation. Different to cow milk, camel milk fat contains few short-chain fatty acids (C4 - C12). The fatty acid pattern contains more long-chain fatty acids (C14, C16 and C18) and resembles to human milk fat (Farah, 1993). The protein content ranges between 2.5 and



4.0 % (Alshaik and Salah, 1994). Similar to the horse and human milk, there is no  $\beta$ -lactoglobulin in camel milk in which  $\alpha$ -lactalbumin is the principal whey protein. The lactose values are reported with 3.8 to 5.7 % (Alshaik and Salah, 1994; Field *et al.*, 1997) which compares to cow or ewe's milk and are little less than human milk. Finally, the ash content is given with 0.7 to 1.2 %. Camel milk is rich (24 - 36 mg/l) in vitamin C compared to cow milk (3 – 23 mg/l). The average pH of camel milk is reported with values between 6.2 and 6.8 (Field *et al.*, 1997; Khaskheli *et al.*, 2005). This average can be compared to the pH of ewe's milk. It can increase up to 7.2 in case of clinical mastitis (Tuteja *et al.*, 2003).

### *Camel milk characteristics*

Camel milk has high antimicrobial activity to inhibit the growth of pathogenic microorganisms when compared to cow milk (Gnan *et al.*, 1998) which can be attributed to compounds that are more active in camel milk whey than in casein. It contains a number of enzymes with anti-bacterial and anti-viral properties (lactoferrin, peptidoglycan recognition protein (PGRP) and Lysozyme). It also contains lactoperoxides that suppress Gram negative bacteria and most effective in raw milk. Additionally, the immunoglobulins and N-acetyl-glucosaminidase (NAGase) possesses antiviral activity (Wernery, 2003). El-Agamy *et al.* (1992) extracted lysozyme, lactoferrin, lactoperoxidase and immunoglobulin A from camel milk. The activity of these protective proteins was assayed against *Lactococcus lactis sub-spp.*, *Cremoruse*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhimurium* and *Rotavirus*.

The antibacterial spectrum of camel milk is similar to that of egg white lysozyme and different from the lysozyme of bovine milk. However, the specific lytic activity of camel milk lysozyme on *Escherichia coli* and *Micrococcus lysodeikticus* is lower than that of lysozyme from the human milk and egg white (Duhaiman, 1988). Bovine and camel milk lactoferrin, antibacterial activity spectra is similar. In camel milk, the activation of the lipopolysacchrides may induce a longer-lasting bacteriostatic effect than in cow's milk due to the presence of higher levels of other indigenous antimicrobial components (Ramet, 2001).



Besides to its nutritional importance, camel milk is known by its medicinal properties, which are widely exploited for human health in several countries. Camel milk is considered to have anti-cancer, hypo-allergic, and anti-diabetic properties. The low quantity of  $\beta$ -casein and the lack of  $\beta$ -lactoglobulin are linked to the hypo-allergic effect of camel milk. Other components such as lactoferrin, immunoglobulins, lysozyme or vitamin C were reported to play a central role in the determination of these properties (Konuspayeva *et al.*, 2009). It was found that one of the camel's milk proteins is similar to insulin (Beg *et al.*, 1986). The authors used a specific antibody to insulin in a RIA test for insulin in camel's milk and discovered large concentrations of insulin: 40 units/l. In India and Asia, camel milk is used therapeutically against dropsy, jaundice, tuberculosis, asthma, anemia, piles besides the problem of spleen (Rao *et al.*, 1970; Sharmanov *et al.*, 1978). The camel milk acts as a laxative on people unaccustomed to drinking this milk. The medicinal importance of camel milk is greater if the camel eat shrubs and bushes than straw. The shrubs and bushes are, themselves, used in the preparation of medicines.

## **2.2. Microbial Quality of Raw Camel Milk**

### **2.2.1. Sources of microbial contamination of raw milk**

Even though raw milk aseptically drawn from a healthy animal usually contains a few bacteria, milk is an ideal medium for the growth of microorganisms from surrounding environment (Pal, 2012). Normally, the first contamination of milk takes place at the moment of milking during the passage of the teat canal and by milking equipment or milking personnel. Further contamination is possible during transport and storage of the milk. According to the report of Semereab and Molla (2001), the main reasons for spoilage of milk were saprophytic microorganisms. Poor management and unhygienic milking practices prevalent in the traditional husbandry systems, which include tying the teats with soft barks to prevent the calf from suckling, tick infestations and cauterization of the udder and skin, are few of the factors responsible for contamination of milk (Obied *et al.*, 1996; Almaw and Molla, 2000).



A recent study conducted in Ethiopia by Hadush *et al.* (2008) in Ab-'Ala district indicated that the major bacterial isolates from udder were pathogenic *S. aureus* (31.82%), *Rhodococcus equi* (22.73%), *Acinetobacter* spp. (18.18%), non pathogenic *Staphylococcus* spp. (9.09%), *Mannheimia haemolytica* (9.09%), *Escherichia coli* (4.54%) and *Alcaligenes* spp. (4.54%). Almost all these organisms were also isolated from milk samples collected from milking vessels with higher incidence rate. But in addition to *S. aureus*, *E. coli*, *Pseudomonas* spp., *Bacillus* spp., and *Alcaligenes* spp. were reported as common microflora of raw milk by Sherikar *et al.* (2004). Hadush and his colleagues (2008) isolated *Pseudomonas* and *Bacillus* only in samples taken from milking vessels and this may indicate that these were the common environmental contaminants. Growth of mesophilic bacteria is found in 60 % of milk samples. Very recently, Pal (2012) described the isolation of *Bacillus cereus*, *Campylobacter jejuni*, *Listeria monocytogenes*, *S. aureus*, *Salmonella* spp. and toxigenic strains of *Aspergillus flavus* from raw milk and milk products of dairy cows.

#### 2.2.2. Mastitis in camel

A high percentage of subclinical mastitis in camels is reported by several authors (Barbour *et al.*, 1985; Guliye, 1996; Obied *et al.*, 1996; Almaw and Molla, 2000). The pathogenic bacteria found by different scientific groups are similar to bacteria reported in mastitis of cows or other animals kept in traditional nomadic environment or camel farms (Barbour *et al.*, 1985; Almaw and Molla, 2000). Two decades ago, there were little reports of mastitis problem at herd level; but today it is reported from almost all camel rearing countries (Al-Ani and Al-Shareefi, 1997; Bekele and Molla, 2001; Guliye *et al.*, 2002; Khedid *et al.*, 2009; Mohammed *et al.*, 2005). Whether the occurrence of the disease has earlier been underestimated, or an upsurge of the disease took place due to dairying interest remains unclear. *S. aureus*, *M. haemolytica* and *E. coli* have been reported to be responsible for mastitis in camel (Bekele and Molla, 2001). Clinical and subclinical mastitis of camel are found to be prevalent in different parts of Ethiopia. The prevalence of 2.1% and 12.5% were reported by Almaw and Molla (2000) and Bekele and Molla (2001), respectively from eastern part of Ethiopia. Higher prevalence of subclinical mastitis in camel was recorded in Dire Dawa (46.2%) followed by Gewane (Afar Region) 45.4% and 37.1% from Negele Borena (Abdel Gadir, 2001). A prevalence of 22.5% (Almaw and Molla, 2000)



and 19.1% (Bekele and Molla, 2001) were also reported from eastern and north eastern parts of Ethiopia, respectively.

### 2.2.3. Total bacteria count (TBC)

Sources of contamination vary according to the keeping and milking conditions of the camels. Under pastoral production conditions, environmental contamination is likely to play a bigger role in the hygiene of raw camel milk than initial bacterial contamination of the camel milk (Younan, 2004). The TBC of camel milk is reported with values that vary between  $10^2$  and  $10^8$  cfu/ml (Semereab and Molla, 2001; Wernery *et al.*, 2002; Sela *et al.*, 2003; Younan, 2004;). These differences underline the fact that TBC depends on several parameters: like the camel milk itself, contamination of the camel udder and contamination by milking personal, containers, etc. Different average TBC in camel raw milk is summarized in Table 2.

Table 2: Average total bacterial count in camel milk samples

| Country                            | TBC (cfu/ml)                                    | Authors                      |
|------------------------------------|-------------------------------------------------|------------------------------|
| Ethiopia, udder milk sample        | $4 \times 10^5$                                 | Semreab and Molla, 2001      |
| UAE , bowl samples                 | $94.1\% \leq 1.0 \times 10^5$                   | Wernery <i>et al.</i> , 2002 |
| Ethiopia, udder milk sample        | $10 - 4.5 \times 10^5 > 65\% 10^4$              | Mengistu, 2003               |
| Ethiopia, bowl sample              | $6.5 \times 10^2 - 7.5 \times 10^6 > 86\% 10^4$ | Mengistu, 2003               |
| Israel                             | $8.0 \times 10^4 - 5.3 \times 10^8$             | Sela <i>et al.</i> , 2003    |
| Kenya, bucket samples              | $10^3 - 10^5$                                   | Younan, 2004                 |
| Kenya, udder milk sample           | $10^2 - 10^4$                                   | Younan, 2004                 |
| Dubai (UAE), hand milked samples   | $1.1 \times 10^2$                               | Eberlein, 2007               |
| Dubai (UAE) quarter-milk samples   | $9.2 \times 10^2$                               | Eberlein, 2007               |
| Dubai (UAE) machine milked samples | $9.2 \times 10^2$                               | Eberlein, 2007               |
| Sudan                              | $1.8 \times 10^5$                               | Omer and Eltinay, 2008       |



#### 2.2.4. *Staphylococcus* species

Staphylococci are small Gram-positive cocci belonging to the family of *Micrococcaceae*. The species can be subdivided into two groups showing either coagulase positive or coagulase negative reactions. These bacteria are the most frequently isolated from camels and can be considered as main reason for subclinical mastitis in dromedaries (Abdel Gadir *et al.*, 2005; Almaw and Molla 2000; Sena *et al.*, 2000; Obied *et al.*, 1996). Omer and Eltinay (2000) reported a total *Staphylococcus aureus* count of  $1.2 \times 10^3$  cfu/ml.

The prevalence of staphylococci varies according to the different studies, but there is nearly no investigation on the bacteriological hygiene of camel milk where staphylococci are not mentioned (Eberlein, 2007). The prevalence of CPS is given with 0.5 - 32 % in samples from clinically healthy camels (Omer and Eltinay, 2008; Wernery *et al.*, 2002) and up to 50 % in cases of clinical mastitis (Hafez *et al.*, 1987). CNS are reported with a prevalence of 3.6 to over 56 % of the samples in clinically inapparent udders (Abdel Gadir *et al.*, 2005; Almaw and Molla, 2000) and with 13 % in cases of clinical mastitis (El-Jakee, 1998). In most investigations both CNS and CPS were isolated from the milk of the same camels. An overview on the prevalence of staphylococci in camel milk reported by many investigators is given Table 3.

The term “CPS” generally refers for *Staphylococcus aureus*. Other CPS like *S. intermedius* or *S. hyicus* may occur in camel milk (Abdel Gadir *et al.*, 2005). In cow and goat milk, these bacteria apparently do not play an important role in milk and milk products. Several investigators from different parts of the world including Ethiopia mentioned that *S. aureus* is the main cause of chronic mastitis in camels (Barbour *et al.*, 1985; Hafez *et al.*, 1987; Al-Ani and Al-Shareefi, 1997; El-Jakee, 1998; Eberlein, 2007; Haduash *et al.*, 2008). Similarly, Guliye, 1996; Chaffer *et al.*, (2000) and Tuteja *et al.* (2003) observed a clearly increased somatic cell count in milk in which *S. aureus* was proved and considered as a main cause for clinical and subclinical mastitis in camel. The most often isolated CNS from camel milk is *S. epidermidis* (Tuteja *et al.*, 2003; Abdel Gadir *et al.*, 2005).



Table 3: Prevalence of staphylococci in camel milk

| Author(s)                         | Country      | <i>S.aureus</i> (%) | CNS (%) | Samples (n) | Camels (n) |
|-----------------------------------|--------------|---------------------|---------|-------------|------------|
| <b>Healthy camels</b>             |              |                     |         |             |            |
| Barbour <i>et al.</i> , 1985      | Saudi Arabia | 17.1                | -       | 205         | 205        |
| Saad and Thabet, 1993             | Egypt        | 5.9                 | -       | 40          | 40         |
| Obied <i>et al.</i> , 1996        | Sudan        | -                   | 11.7    | 757         | 757        |
| Guliye, 1996                      | Israel       | X                   | -       | 86          | 86         |
| El-Jakee, 1998                    | Egypt        | 5.0                 | 10.0    | 100         | 100        |
| Almaw and Molla, 2000             | Ethiopia     | 0.6                 | 3.6     | 753         | 195        |
| Chaffer <i>et al.</i> , 2000      | Israel       | 8.8                 | 20.4    | 137         | 35         |
| Younan <i>et al.</i> , 2001       | Kenya        | 11.0                | -       | 1242        | 207        |
| Semreab and Molla, 2001           | Ethiopia     | 14.9                | 31.7    | 130         | 130        |
| Wernery <i>et al.</i> , 2002      | UAE          | 0.5                 | -       | 1313        | 14         |
| Mengistu, 2003                    | Etiopia      | 7.8                 | 17.7    | 141         | -          |
| Tuteja <i>et al.</i> , 2003       | India        | 20.9                | 27.8    | 282         | 71         |
| Abdel Guadir <i>et al.</i> , 2005 | Ethiopia     | 24.6                | >56     | 956         | 253        |
| Eberlein, 2007                    | UAE          | 5.4                 | -       | 196         | 18         |
| Omer and Eltinay, 2008            | Sudan        | 32                  | -       | -           | 50         |
| <b>Subclinical mastitis</b>       |              |                     |         |             |            |
| Sena <i>et al.</i> , 2000         | India        | 14.0                | -       | 150         |            |
| <b>Mastitis cases</b>             |              |                     |         |             |            |
| Hafez <i>et al.</i> , 1987        | Saudi Arabia | 50.0                | -       | 62          | 62         |
| El-Jakee, 1998                    | Egypt        | 17.0                | 13.0    | 100         | 100        |



#### 2.2.5. *Streptococcus* species

*Streptococcus* spp. is reported in most articles in connection with the hygiene of camel milk. *Streptococcus agalactiae*, *S. dysagalactiae* and *S. uberis* were the main isolates that are found in camel milk (Table 4). *S. agalactiae* is reported as one of the main cause for clinical mastitis in camels and a potential human pathogen, causing infections mainly in new borns (Younan, 2004; Almaw and Molla, 2000). In Ethiopia, Abdel Gadir *et al.* (2005) isolated 7.0 % *S. uberis* out of 956 quarter milk samples, of which 95 % were taken from cases of subclinical mastitis and 5 % of clinical mastitis.



Table 4: *Streptococcus* species in camel milk

| Author(s)                        | Country  | <i>Streptococcus</i><br><i>spp.</i> (%) | <i>S.</i><br><i>agal</i><br><i>actiae</i><br>(%) | <i>S.</i><br><i>disagalactae</i><br>(%) | Sample<br>(n) | Camel<br>(n) |
|----------------------------------|----------|-----------------------------------------|--------------------------------------------------|-----------------------------------------|---------------|--------------|
| <b>Healthy camels</b>            |          |                                         |                                                  |                                         |               |              |
| Barbour <i>et al.</i> , 1985     | S Arabia | 4.9                                     | -                                                | -                                       | 205           | 205          |
| Guliye, 1996                     | Israel   | -                                       | -                                                | X                                       | 86            | 86           |
| Al-Ani and Al-Shareefi,<br>1997  | Iraq     | X                                       | -                                                | -                                       | 50            | -            |
| El-Jakee, 1998                   | Egypt    | 12.0 (S.uberis<br>4.0)                  | 8.0                                              | -                                       | 100           | 100          |
| Chaffer <i>et al.</i> , 2000     | Israel   | 1.5                                     | -                                                | -                                       | 137           | 35           |
| Almaw and Molla, 2000            | Ethiopia | -                                       | 1.5                                              | -                                       | 753           | 195          |
| Younan <i>et al.</i> , 2001      | Kenya    |                                         | 12                                               | -                                       | 1242          | 207          |
| Semreab and Molla,<br>2001       | Ethiopia | 7.8                                     | 1.8                                              | 1.2                                     | 130           | 130          |
| Tuteja <i>et al.</i> , 2003      | India    | 20.9                                    | 10.4                                             | 10.4                                    | 282           | 71           |
| Mengistu, 2003                   | Ethiopia | 18.4                                    | 15.2                                             | 2.8                                     | 141           | -            |
| Abdel Gadir <i>et al.</i> , 2005 | Ethiopia | S. uberis 7.0                           | 26                                               | -                                       | 956           | 253          |
| <b>Subclinical mastitis</b>      |          |                                         |                                                  |                                         |               |              |
| Sena <i>et al.</i> , 2000        | India    | -                                       | 67.4                                             | -                                       | 150           | -            |
| <b>Mastitis cases</b>            |          |                                         |                                                  |                                         |               |              |
| El-Jakee, 1998                   | Egypt    | 28.0 (S. uberis<br>12.0)                | 16.0                                             | -                                       | 100           | 100          |
| Hafez <i>et al.</i> , 1987       | S Arabia | 33.3                                    | -                                                | -                                       | 62            | 62           |

X Presence

2.2.6. Coliforms

Coliforms are a heterogeneous group of *Enterobacteriaceae* (*E. coli*, *Klebsiella*, *Enterobacter*, lactose positive biotypes of *Citrobacter*, *Serratia* and *Hafnia*). A high percentage of biotypes of these species originate from the soil or water, some come from faecal contamination. Mengistu (2003) reported a prevalence of 17.4% in composite camel milk samples. Al-Mohizea (1986) and Omer and Eltinay (2008) reported a total coliform count of  $6.8 \times 10^1$  and  $5.1 \times 10^5$  cfu/ml from camel milk, respectively. Coliforms, besides *E. coli*, that are reported in camel milk are *Klebsiella pneumoniae* (0.5 – 8.3 % of the milk samples) and *Citrobacter freundii* (0.6 – 11.8%). In most cases, these bacteria are present in clinically healthy camel udders, whereas one author isolated these bacteria from cases of severe mastitis as indicated in Table 5.

Table 5: Coliform species recovered from camel milk

| Author(s)                        | Country        | <i>Citrobacter<br/>freundii</i> (%) | <i>Klebsiella<br/>pneumoniae</i> (%) | Sample<br>(n) | Camels<br>(n) |
|----------------------------------|----------------|-------------------------------------|--------------------------------------|---------------|---------------|
| <b>No clinical mastitis</b>      |                |                                     |                                      |               |               |
| Barbour <i>et al.</i> , 1985     | Saudi Arabia   | -                                   | 0.5                                  | 205           | 205           |
| El-Jakee, 1998                   | Egypt          | 3.0                                 | 2.0                                  | 100           | 100           |
| Semreab and Molla, 2001          | Ethiopia       | 0.6                                 | 7.1                                  | 130           | 130           |
| Mengistu, 2003                   | Ethiopia       | 0.7                                 | 4.4                                  | 141           | -             |
| Abdel Gadir <i>et al.</i> , 2005 | Ethiopia       | -                                   | 0.9                                  | 956           | 253           |
| Eberlein, 2007                   | UAE<br>(Dubai) | 11.8                                | 8.3                                  | 260           | 25            |
| <b>Clinical sign of mastitis</b> |                |                                     |                                      |               |               |
| El-Jakee, 1998                   | Egypt          | -                                   | 100                                  | 1             | 1             |

Coliforms have been reported in camel milk from several authors - mainly in clinically healthy udders. In milk samples taken from healthy camels, the prevalence of *E. coli* ranges between 1.0 and 17.3 % (El-Jakee, 1998; Eberlein, 2007) and that of coliforms from 1.4 % up to 29.4 % (Saad



and Thabet, 1993; Wernery *et al.*, 2002). In addition, Abdel Gadir *et al.* (2005) isolated *E. coli* mainly (99.0 % of the isolates) from camel quarters that showed signs of subclinical mastitis. They also reported one case of clinical mastitis caused by *E. coli*. This bacterium (*E. coli*) is also known as pathogenic bacteria causing severe intestinal and extraintestinal diseases in man (Kaper *et al.*, 2004; Pal, 2007) as well as mastitis in cows (Bradley and Green, 2001). A peracute case of mastitis in she-camels due to *E. coli* following a caesarean section was reported. Moreover, both groups are known as mastitis pathogens in cows. The prevalence of *E. coli* and coliforms in camel milk is given in Table 5. Coliforms and *E. coli* are often used as marker organisms. The presence of coliforms is usually used as an indicator for poor manufacturing hygiene, and that of *E. coli* is a marker for faecal contamination due to the fact that it is a commensal of the intestinal tract.

Table 6: Prevalence of coliforms and *E. coli* in camel milk

| Author(s)                        | Country      | Coliforms<br>(%) | <i>E. coli</i><br>(%) | Samples<br>(n) | Camels<br>(n)   |
|----------------------------------|--------------|------------------|-----------------------|----------------|-----------------|
| <b>Healthy camels</b>            |              |                  |                       |                |                 |
| Barbour <i>et al.</i> , 1985     | Saudi Arabia | -                | 1.5                   | 205            | 205             |
| Saad and Thabet, 1993            | Egypt        | 29.4             | -                     | 40             | 40              |
| Guliye, 1996                     | Israel       | -                | -                     | 86             | 86              |
| Al-Ani and Al-Shareefi, 1997     | Iraq         | -                | -                     | 50             | 50              |
| El-Jakee, 1998                   | Egypt        | 3.0              | 1.0                   | 100            | 100             |
| Semreab and Molla, 2001          | Ethiopia     | X                | 8.3                   | 130            | 130             |
| Wernery <i>et al.</i> , 2002     | UAE          | 1.4              | -                     | 1313           | 14              |
| Mengistu, 2003                   | Ethiopia     | 6.4              | 16.5                  | 46             | -               |
| Abdel Gadir <i>et al.</i> , 2005 | Ethiopia     | -                | 17.3                  | 95             | 250             |
| Eberlein, 2007                   | UAE(Dubai)   | 6.5              | 23.5                  | 260            | 25 <sup>1</sup> |
| <b>Subclinical mastitis</b>      |              |                  |                       |                |                 |
| Sena <i>et al.</i> , 2000        | India        | -                | 9.3                   | 150            | -               |
| <b>Mastitis</b>                  |              |                  |                       |                |                 |
| El-Jakee, 1998                   | Egypt        | 11.0             | 6.0                   | 100            | 100             |

<sup>x</sup> Presence

<sup>1</sup> Machine milked samples



Cattle, besides other ruminants, are considered as the main reservoir for Verotoxinogenic *Escherichia coli* (VTEC) as they excrete *E. coli* mostly without showing symptoms. Outbreaks through consumption of raw milk, pasteurised milk and raw milk cheese are reported (Sayed and Abdel-Hafez, 2005). VTEC infections through sheep or goat milk have rarely been reported. WHO (2005a) mentioned that the camel may also act as reservoir for VTEC serotype O157:H7. However, other reports about camel infection are not available.

#### 2.2.7. *Bacillus cereus*

*Bacillus cereus*, which is a Gram-positive facultative anaerobe rod, is one of the causative agents for acute mastitis in cows and is often found in milk. Eberlein (2007) reported the presence of *B. cereus* in camel milk with a prevalence of 3.4 % from Dubai. In Ethiopia, the presence of *B. cereus* in camel milk is reported by Abdel Gadir *et al.* (2005) with a prevalence of 9.1 % of the 956 quarter milk samples taken from 253 traditionally managed lactating camels. The prevalence (6.5 %) of *B. cereus* is described by Mengistu (2003) from composite milk samples from Somali region. In Egypt, Saad and Thabet (1993) also reported the presence of *B. cereus* in the milk samples of healthy camels with a prevalence of 29.4 %. *B. cereus* is the cause of two different types of foodborne diseases in man: a diarrhoeal type due to the production of enterotoxins in the small intestine and an emetic type, which is caused by the ingestion of a toxin produced in the foodstuff.

#### 2.2.8. *Salmonella* species

*Salmonella* spp. belongs to the family *Enterobacteriaceae*. They are Gram-negative, facultative anaerobe rods with more than 2500 known serovars (Pal, 2007). *Salmonella* infection in camels has been reported in various countries, including Ethiopia, Sudan, Egypt, Somalia, USA and UAE and *S. Typhimurium* and *S. Enteritidis* are more prevalent in camels (Matofari *et al.*, 2007). Healthy camels can be carriers of *Salmonella*, and organisms have been isolated from faeces and lymphnodes on slaughter of camels (Wernery, 1992). The most frequent reason for the presence of *Salmonella* spp. in milk is through faecal contamination after heat treatment as salmonellae are



inactivated during pasteurization. *Salmonella* spp. are of high importance in food safety being able to provoke severe intestinal infections in humans which can lead to death especially in elderly people (WHO, 2005b). According to the study conducted by Matofari *et al.* (2007), 43% of 196 composite milk samples were found to contain *Salmonella* spp. Out of these, 31% were positively identified as *S. enterica*.

#### 2.2.9. *Listeria* species

Listeriae are Gram-positive widespread rods with a high resistance against environmental influences as cold, drought and solar radiation and are growing well in cold environment. From the genus *Listeria*, mainly *Listeria monocytogenes* is of health importance for animals and humans (Pal, 2007). The most common symptoms of listeriosis caused by *L. monocytogenes* are the dysfunction of the central nervous system, abortion and diarrhoea with possible lethal outcome, especially in predisposed individuals like pregnant women, children, elderly and immunosuppressed people. Other species such as *L. ivanovii* and *L. seeligeri* are of minor importance or are considered as non pathogenic as *L. innocua*. Very few reports are available about *Listeria* infections in old world camels. But a serological positive reaction was observed in 34.6 % of the tested camels in Tunisia. A recent study conducted in Iran by Rahimi *et al.*, (2010) reported the presence of *Listeria* in camel milk with a prevalence of 2.1 % (1/48). On the other hand, Omer and Eltinay (2008) reported that they could not find *Listeria* from camel milk samples.

#### 2.2.10. Other bacteria

In addition to the above mentioned bacteria, other species are also reported. An overview of these microorganisms isolated from camel milk is indicated in Table 8. The prevalence of *M. haemolytica* is given with 1.5 - 6.0 % by six out of seven authors in the milk of clinically inapparent camels and with 3.0 and 16.7% by two authors (El-Jakee, 1998; Hafez *et al.*, 1987) in samples of camels with clinical signs of mastitis. *Pseudomonas aeruginosa* was isolated by four of seven authors from healthy camels with a prevalence of 1.0 - 17.7 %. No isolation of bacteria from mastitic milk of camel is reported. Six of seven authors detected *Micrococcus* spp. in the



milk of clinically inapparent udders with a prevalence of 0.5 - 25.4 %, whereas one of six authors (El-Jakee, 1998) found these bacteria in 19 % of the milk samples obtained from mastitis cases. The prevalence of *Arcanobacterium pyogenes* was recorded from 1.0 - 10.0 % in milk of clinically inapparent camels by four authors and 2.0 % in samples of camels with mastitis by El-Jakee (1998).

El-Jakee (1998) also isolated anaerobic bacteria such as *Clostridium perfringens* (56 % toxigenic), *Peptostreptococcus* spp. and *Fusobacterium necrophorum*, both from inflamed udders and apparently healthy udders of camels.



Table 7: Other bacterial findings in camel milk

| Author/s                             | Country   | Samples (n) | Camels (n) | <i>Micrococcus spp.</i> (%) | <i>M. haemolyticus</i> (%) | <i>M. aeruginosa</i> (%) | <i>A. pyogenes</i> (%) |
|--------------------------------------|-----------|-------------|------------|-----------------------------|----------------------------|--------------------------|------------------------|
| <b>Healthy camels</b>                |           |             |            |                             |                            |                          |                        |
| Barbour <i>et al.</i> , 1985         | S. Arabia | 205         | 205        | 25.4                        | 1.5                        | 1.0                      | 1.0                    |
| Saad and Thabet, 1993                | Egypt     | 40          | 40         | 23.5                        | -                          | 17.7                     | -                      |
| El-Jakee, 1998                       | Egypt     | 100         | 100        | 17.0                        | 6.0                        | 5.0                      | 10.0                   |
| Almaw and Molla, 2000                | Ethiopia  | 753         | 195        | X                           | x                          | -                        | X                      |
| Semreab and Molla, 2001              | Ethiopia  | 130         | 130        | -                           | 5.4                        | 4.8                      | 1.2                    |
| Mengistu, 2003                       | Ethiopia  | 141         | -          | -                           | 2.2                        | 7.8                      | 6.5                    |
| Abdel Gadir <i>et al.</i> , 2005     | Ethiopia  | 956         | 253        | 0.5                         | 2.1                        | -                        | -                      |
| <b>Camels with clinical mastitis</b> |           |             |            |                             |                            |                          |                        |
| Hassanein <i>et al.</i> , 1984       | Egypt     | 1           | 1          | -                           | -                          | -                        | X                      |
| Hafez <i>et al.</i> , 1987           | S. Arabia | 62          | 62         | -                           | 16.7                       | -                        | -                      |
| El-Jakee, 1998                       | Egypt     | 100         | 100        | 19.0                        | 3.0                        | -                        | 2.0                    |

x presence



### 2.2.11. Moulds and Yeasts

The role of fungi in the etiology mastitis in dairy animals is well recognized (Pal and Mehrota 1983; Pal and Lee, 1991). Among various mycotic pathogens, *Cryptococcus neoformans* produce a severe type of mastitis (Pal and Randhawa, 1976).

El-Jakee (1998) found the presence of *Candida albicans* in 4 % of camels with clinical signs of mastitis. In none of the healthy udders, *Candida albicans* was found. Omer and Eltinay (2008) found total yeast and mold count of  $4.1 \times 10^{-1}$  cfu/ml, and out of 50 milk samples, 15% were positive for yeast. A study was conducted in 2007 by El. Ziney and Al-Turki on the microbiological quality and safety assessment of camel milk in Saudi Arabia. The mean log counts per ml for aerobic total plate counts of moulds and yeasts was 1.9 (El-Ziney and Al-Turki, 2007). However, in most investigations on camel milk, no yeasts were detected or were not within the scope of examination.

### 2.3. Public health significance of raw camel milk

Generally, a number of diseases can be considered as milk borne zoonoses. Some of these are infectious hepatitis, poliomyelitis, Q fever, toxoplasmosis, anthrax, brucellosis, campylobacteriosis, colibacillosis, corynebacteriosis, Haver hill fever, listeriosis, salmonellosis, shigellosis, staphylococcosis, streptococcosis, tuberculosis and yersiniosis (Pal, 2007). Most of the causative agents of these diseases are reported from camel milk. As camel milk is usually consumed in its raw state or in varying degrees of sourness in the raw state (without heat treatment), the pathogenic microbes that present in it can pose a health hazard to the consumer (Saad and Thabet, 1993; Younan, 2004).

Like in other animals, mastitis is common in camel. Many mastitis producing pathogens are of public health concern as some of them are capable of producing toxins or causing infections in man (Semereab and Molla, 2001). *S. aureus* was proved and considered to be the main cause for clinical and subclinical mastitis. Mastitis in milking dromedary is not only of veterinary interest but represents a direct threat to human health (Younan, 2004). This is because that *S. aureus* can



produce heat stable enterotoxins that are not inactivated during pasteurization of milk or production of milk products and can provoke food intoxication (vomiting and diarrhea). One of the main risk factors for production of staphylococcal enterotoxin is the storage of milk at ambient temperature after milking. Already a short storage time can lead to enhanced growth of CPS and can present a serious problem to human health if the strain produces enterotoxin (Pal, 2001).

The main reasons for spoilage of milk are saprophytic microorganisms. The handling of informally marketed milk has been reported to affect the safety and quality of milk with reports of food poisoning due to consumption of camel milk also being reported (Matofari *et al.*, 2007). Currently, VTEC is considered as the main food borne causative agents of human intestinal infections. Mainly the serovar O157:H7 provoked several outbreaks in Northern America, Europe, Japan, and other countries. Beside some other factors the principal virulence factor of VTEC is the production of toxins (shiga toxins or verotoxins).

*Brucella abortus*, *B. melitensis* and *B. ovis* can cause brucellosis in camel. Camel brucellosis is found to be prevalent in Afar (5.2%), in Somali (2.8%), and Borena (1.8%) areas of the country (Bekele, 2004). Raw milk from infected animals provides a risk for brucellosis for the consumers (Acha and Szyfres, 2001; Pal, 2007).



### 3. MATERIALS AND METHODS

#### 3.1. Description of the Study Area

The study was conducted in Fentale District of Oromiya region from August 2011 to April, 2012. Fentale is one of the 180 woredas in the Oromia Region of Ethiopia. The administrative center of Fentale District is Metehara. Fentale is bordered on the southeast by the Arsi Zone, on the southwest by Boset, on the northwest by the Amhara Region, and on the northeast by the Afar Region. Fentale District is located at about 198 kms east of Addis Ababa and lies at 8° 54' North latitude and 36° 23' to 39° 54' East longitude. The District is situated at an altitude of 650 to 1010 meters above sea level. The average annual rainfall of the district is 486 mm. It has a mean minimum and maximum temperature of 36 °C and 42 °C, respectively (FARM Africa Pastoralist Livelihood Initiative Project, Two Years Report, 2005-2007).

A survey of the land in this Woreda showed that 8.2 % is arable or cultivable, 7.6 % pasture, 28.8 % forest, and the remaining 55.4 % is considered degraded or otherwise unusable. The vegetation is primarily acacia trees with bushes and shrubs common to the lowland portions of Ethiopia. In 11 of the 18 kebeles of Fentale, the predominant agricultural practice is pastoralism. The livestock population in East Shoa Zone is about 865,106 cattle, 347,050 sheep, 549,993 goats, 13,809 horses, 8,282 mules, 194,083 donkeys, 68,331 camels and 1,015,328 poultry. Camels, goats and cattle are the most common livestock; migration to the border areas of Boset Woreda for grazing during normal years is common, but in years of low rainfall herdsmen migrate as far as Negele Arsi (PCDP, 2004).

Based on figures published by the Central Statistical Agency in 2005, this Woreda has an estimated total population of 87,424, of whom 41,437 are men and 45,987 are women; 21,348 or 24.42% of its population are urban dwellers. With an estimated area of 1,169.85 square kilometers, Fentale has an estimated population density of 74.7 people per square kilometer (PHCE, 1994). The primary ethnic groups reported in this Woreda included the Karayu and Ittu Oromos.



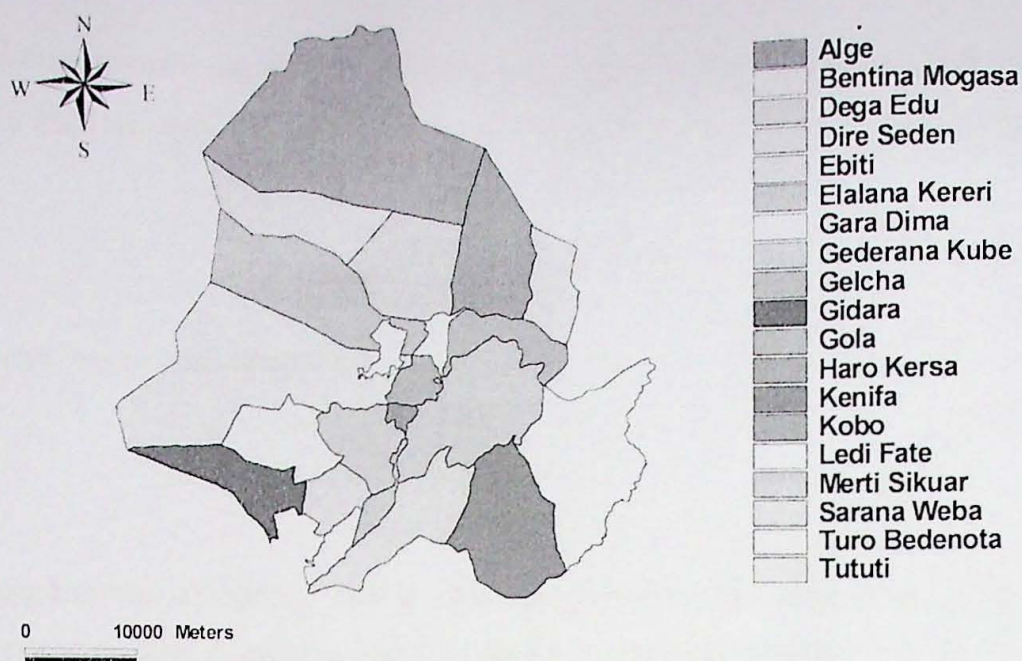


Fig 1: Fentale Woreda kebele location map

### 3.2. Study design

A cross sectional study was conducted from August 2011 to April 2012 to isolate and identify species of bacteria, and to assess bacterial load of raw camel milk samples in the study areas.

### 3.3. Study population

All lactating one humped camels in the study areas (Tututi, Elanana kereri and Gelcha) were considered as a study population. The milk samples were collected from these lactating camels.



### 3.4. Sample size determination

The approximate sample size required was determined using an estimated prevalence of 50% for each bacterial spp., defined precision of 5% and 95% level of confidence (Thrusfield, 2005).

$$n = \frac{1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}$$

Where, n=required sample size

$P_{\text{exp}}$ =expected prevalence

d=desired absolute precision

Therefore, the calculated milk sample size was from 384 camels. In addition, 36 milk samples were obtained from milking utensils, used for collecting of camel milk in the herd. Therefore, the total number of milk samples was 420.

### 3.5. Sampling method

Fentale District consists of 18 kebeles. In 11 of 18 kebeles of Fentale the predominant agricultural practice is pastoralism. Three Pastoral kebeles namely Tututi, Elanana Kereri and Gelcha were selected conveniently based on accessibility by vehicles and proximity to road. From the three pastoral kebeles the lactating camels were sampled randomly from the herd. For the isolation and identification of bacteria, these 384 individual lactating camels as well as 36 milking utensils were sampled. Standard plate count and coliform counts were conducted on 36 milking utensils as well as from 36 camels selected randomly from 384 lactating camels.

### 3.6. Observational study

To determine the general hygiene and possible source of camel milk contamination observation was made on pre-milking udder preparation and hygiene, hygiene of milkers hand, and milking utensils, source of water used for washing milking utensils and methods of storing camel milk,



udder health problems encountered, etc were observed and recorded through an observation checklist (Annex 1).

### **3.7. Examination of udder and milk**

Clinical mastitis was recognized by abnormal milk, signs of udder infection and detection of mastitis pathogens by bacteriological culture, whereas subclinical mastitis was recognized by apparently normal milk and an increase in leukocyte counts as evidenced by California mastitis test (CMT) and a positive culture result. CMT was performed on all milk samples collected from each camel. Two ml of milk was placed in a cup of the paddle to which an equal amount of CMT reagent was added. Then the paddle was rotated gently in a circular motion shortly so that the milk and reagent was mixed well. The CMT reaction was immediately scored and interpreted according to Schalem *et al.* (1971). CMT scores T, 1+, 2+ and 3+ was considered as positive indicators of subclinical mastitis while 0 CMT score indicated absence of subclinical mastitis (Annex 2).

### **3.8. Bacteriological analysis**

#### **3.8.1. Sample collection**

A total of 420 raw milk samples were collected for bacteriological investigation from 384 individual lactating camels and 36 milking utensils. Representative samples were obtained from all quarters (except the blind ones) of the lactating camels and milking utensils. The first squirts of milk were discarded and enough amount of composite milk samples were collected in labeled sterile glass bottles from each of the four quarters. The milk samples from milking utensils were also collected in sterile glass bottles. The milk samples were then kept in an ice box and transported to Microbiology Laboratory of the Collage of Veterinary Medicine and Agriculture, Debre Zeit for examination. The samples were stored at + 4<sup>0</sup> c until processed and analyzed next day.





Fig 2: Collection of milk from the teats of camel in sterilized glass bottle



Fig 3: Collection of milk in a sterilized glass bottle from the dirty plastic container

### 3.8.2. Quantitative analysis of milk for bacteria

Decimal dilutions of milk samples were inoculated on plate count agar (Oxoid, England) and MacConkey agar (Merck, Germany) for standard plate and coliform counts, respectively following the standard procedures recommended by American Public Health Association (1992). Each plate was marked with sample number, before shaking samples and making dilutions.



The procedures followed for standard plate and coliform counts as follows.

(i) Pre-test considerations for milk samples:

Before removal of the milk samples from the containers, the contents were mixed thoroughly and vigorously. The following dilution standards were selected so that the total number of colonies per plate fluctuates between 25 and 250 for standard plate count and between 10 and 100 for coliform counts. Standard plate counts (SPCs) were conducted on udder milk and milk from milking utensils diluted to  $10^{-1} - 10^{-3}$  and  $10^{-1} - 10^{-5}$ , respectively. Whereas coliform counts were conducted on udder milk and milk from milking utensils diluted to  $10^0 - 10^{-2}$  and  $10^0 - 10^{-3}$ , respectively.

While transferring raw milk samples from one test tube to another, sterile pipettes were used. Separate sterile pipettes were used for the different dilutions. Three and five sterile dilution tubes with 9 ml of sterile saline water in them were used for SPCs of raw milk samples from the lactating camels and milking utensils, respectively. On the other hand, two and three sterile dilution tubes with 9 ml of sterile saline water in them were used for coliform counts of raw milk samples from the lactating camels and milking utensils, respectively.

(ii). Dilution and culturing milk samples:

The dilution saline water was labeled with sample number and dilution number. One ml of the milk sample was added to dilution blanks and serially diluted by taking one ml to the next dilution bottle after mixing. One ml was discarded from the last dilution. After thorough mixing, 1 ml of diluted milk was taken from each dilution starting from the highest dilution and put on sterile labeled Petri dishes using sterile pipettes. The covers of the Petri dishes were lifted just high enough to insert pipettes. Two plates were inoculated per dilution. About 10-12 ml of the melted ( $44^{\circ}\text{C}$  to  $46^{\circ}\text{C}$ ) plate count agar and MacConkey agar for SPC and coliform counts, respectively were poured into each plate by lifting gently the cover of the Petri dish just enough to pour the medium. The medium was gently mixed with the test portions in the Petri dish by



rotating and tilting. After the mixture was evenly spread over the bottom of the plate, it was allowed to solidify on a level surface. The plates were then inverted and placed in an incubator at 37<sup>0</sup> C for 48 hours. Incubating control plates for each sterilization, lot of dilution blanks and medium were used to check sterility of the dilution water and medium (APHA, 1992).

(iii). Reading and interpretation of results:

Standard plate count (SPC): After incubation at 37<sup>0</sup> C for 48 hours, all colonies including those of pin point size were counted on selected plates. Results from plates, which contained 25 to 250 colonies per plate were recorded. If plates from two consecutive decimal dilutions yielded colony counts of 25 to 250, the counts for each dilution were computed by the following formula (APHA, 1992).

$$N = \frac{\sum \text{colonies}}{[(1 \times n_1) + (0.1 \times n_2)d]}$$

Where: N= number of colonies per milliliter of milk,  
∑ colonies= sum of colonies on plates counted,  
n<sub>1</sub>= number of plates on lower dilution counted,  
n<sub>2</sub>= number of plates in next higher dilution counted and  
d= dilution from which the first counts are obtained.

When plates contained less than 25 colonies, the results were read as less than 25 times the reciprocal of the dilution number. If more than 250 colonies developed on the highest dilution plates, the count was recorded as more than 250 times the reciprocal of dilution (APHA, 1992).

Coliform count for milk: After incubation of the plate for 48 hours, purplish red colonies with bile precipitation around them were counted as coliforms from MacConkey agar. Results from only those plates, which contained between 10 and 100 colonies were recorded. Interpretations were similar with that of SPC.



### 3.8.3. Qualitative analysis of milk for bacteria

Each milk sample was mixed thoroughly, and a loopfull of milk samples were streaked on to blood agar base enriched with 7 % heparinized sheep blood and MacConkey agar. The plates were aerobically incubated at 37<sup>0</sup> C and examined for bacterial growth after 48 hours. From culture positive plates, typical colonies were subjected to Gram's stain to study the staining properties and cellular morphology. Pure cultures of a single colony type from the blood agar were transferred onto nutrient agar plate. From this, a series of biochemical tests that aided final identification of various bacteria were conducted following standard methods (Quinn *et al.*, 1994) (Annex 2).

*Staphylococcus* spp. and *Micrococcus* were identified based on colonial morphology on blood agar, hemolysis pattern, cellular morphology on Gram's staining, catalase production, coagulase test, O-F test, growth and fermentation on Mannitol salt agar and purple agar. *Streptococcus* spp. and *Enterococcus* spp. were identified based on Gram's reaction, catalase production, hemolysis pattern, differential growth characteristics on Edward's medium and CAMP test. *Corynebacterium* spp. and *Actinomyces* spp. were identified based on staining morphology, hemolytic characteristics, catalase production and sugar fermentation. Gram negative isolates were cultured on MacConkey agar and further identification was made by oxidase reaction, catalase test, the IMViC test (Indol, Methyl red, Vogues- prouskauer and citrate utilization tests), urease and motility test (Annex 3, 4, 5).

### 3.9. Data management and processing

Data were stored in Microsoft excel 2007 spread sheet and transferred to SPSS for statistical analysis. Descriptive statistics such as means, proportions, standard deviations and 95 % confidence intervals were performed. Standard plate count and coliform count were counted as CFUs per ml; the values were transformed into log<sub>10</sub> CFU/ml to normalize the data. The difference in bacterial load between the milk samples from the lactating camels and milking utensils were analyzed using paired t-test. A P-value of < 0.05 was considered indicative of a



statistically significant difference. Percentages were determined by dividing the number of positive samples to the total number of samples examined.



## 4. RESULTS

### 4.1. Observational study

According to observation made on camel milking and milk handling procedures practiced in the study area, the following points were recorded. The pre-milking udder preparation and teat dipping were not practiced in the study area. Due to scarcity of water, washing of the udder before milking, washing of the hands as well as milking utensils with clean water before milking was not practiced. However, most of the animal raisers used urine of camel for cleaning of hands and utensils (Fig. 4, 6 and 7). Very rarely, milk utensils were washed and smoked with well water and wood burning, respectively.

Camel milking was practiced predominantly by males. Due to the height of the camel, milking was always done in standing position with one knee flexed to support the milking utensil (Fig 5). In most of the cases, the dam was milked in the presence of the calf in order to initiate the let down of milk. Sometimes, when the calf was still born or died after birth, the skin of the calf was shown to the dam to induce milking. Hand milking was practiced in the study area (Fig 9). An individual camel-herder milked an average of 15 camels continuously without washing hands between milking of any two camels. For consumption of the milk by the family members, the milking was done four to five times. However, for the purpose of selling the milk, an animal was milked twice a day. The surplus milk was sold in town.

Mixing of milk from sick animal to the milk of health animal and consumption of raw milk was most commonly observed practice in the study area (Fig 10). In addition, there was lack of knowledge about public health hazards of drinking raw milk among the camel-herding society.





Fig 4: Animal with dirty udder used for milk collection



Fig 5: Milking practice followed for collection of milk



Fig 6: Camel raiser milking the animals with dirty hands



Fig 7: Animal keeper touching milk container with dirty hands



Fig 8: Collection of milk by camel owner in unclean plastic container

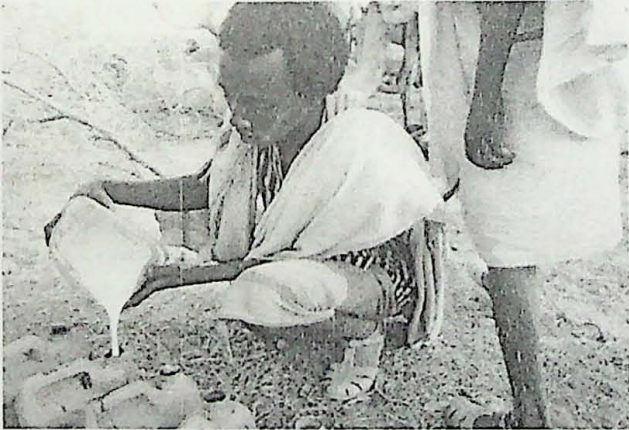


Fig 9: After collection of the milk in dirty container, the milk was poured into dirty plastic jerry-cans





Fig 10: A young boy of nomad family drinking raw camel milk from a dirty plastic container

#### **4.2. Occurrence of mastitis**

##### **Clinical mastitis**

The prevalence of clinical mastitis was determined by clinical examination and culture. Clinically affected udders were swollen, hard and painful upon palpation. From a total 384 lactating camels examined, 16 (4.17 %) were found to be affected with clinical cases of mastitis.

##### **Sub-clinical mastitis**

The prevalence of sub-clinical mastitis was determined based on the results of the CMT and culturing. Based on CMT, the prevalence of sub-clinical mastitis was found to be 79 (20.57 %).



### 4.3. Quantitative analysis of milk for bacteria

#### 4.3.1. Standard plate count (SPC) ( $\text{Log}_{10}$ CFU/ml)

The mean of SPCs of milk samples collected from lactating camels and milking utensils were 4.30 ( $\text{log}_{10}$  CFU/ml) (SD= 0.87) (Range 2.38- 5.53) and 5.34 ( $\text{log}_{10}$  CFU/ml) (SD= 0.72) (Range 4.38-7.18), respectively.

Table 8: Descriptive statistical values ( $\text{log}_{10}$  CFU  $\text{ml}^{-1}$ ) of different SPC detected in milk samples

| Type of sample   | Number of sample | Mean ( $\text{log}_{10}$ CFU $\text{ml}^{-1}$ ) | 95% CI for the mean | SD   | Min. | Max  | T-test | Df | P-value |
|------------------|------------------|-------------------------------------------------|---------------------|------|------|------|--------|----|---------|
| Udder            | 36               | 4.30                                            | 4.03, 4.57          | 0.87 | 2.38 | 5.53 | -9.62  | 35 | 0.000   |
| Milking utensils | 36               | 5.34                                            | 5.11, 5.58          | 0.72 | 4.38 | 7.18 |        |    |         |

CI = confidence interval, SD = standard deviations

Mean difference for SPC of milk samples collected from lactating camels and milking utensils were compared using paired t-test. Statistically significant difference was observed between means of SPC ( $P=0.000$ ) milk samples from lactating camels and milking utensils (Table 9).



Table 9: Paired comparison of mean difference in total SPC from lactating camels and milking utensils

| Sample types | Sample Size | *Mean of the differences | SD   | 95% CI       | T-test | Df | P-value |
|--------------|-------------|--------------------------|------|--------------|--------|----|---------|
| Udder-MU     | 72          | -1.04                    | 0.65 | -1.26, -0.82 | -9.62  | 35 | 0.000   |

\*  $\text{Log}_{10}\text{CFUml}^{-1}$  values, MU= milking utensils, SD= standard deviation of the mean difference, CI= confidence level of the mean difference, df = degree of freedom

#### 4.3.2. Coliform count (Log 10 CFU/ml)

The mean of coliform count of milk samples obtained from lactating camels and milking utensils were 0.98 ( $\log_{10}$  CFU/ml) (SD= 1.35) (Range < 1-4.18) and 3.57 ( $\log_{10}$  CFU/ml) (SD= 0.98) (range < 1- 4.93), respectively.

Table 10: Descriptive statistical values ( $\log_{10}$  CFU/ml) of different coliform count detected in milk samples

| Type of sample   | Number of sample | Mean ( $\log_{10}$ CFU $\text{ml}^{-1}$ ) | 95% CI for the mean | SD   | Min. | Max  | T-test | df | P-value |
|------------------|------------------|-------------------------------------------|---------------------|------|------|------|--------|----|---------|
| Udder            | 36               | 0.98                                      | 0.54-1.42           | 1.35 | < 1  | 4.18 | -13.13 | 35 | 0.000   |
| Milking utensils | 36               | 3.57                                      | 3.25-3.89           | 0.98 | < 1  | 4.93 |        |    |         |

CI = confidence interval, SD = standard deviations



Mean difference for coliform count of milk samples obtained from lactating camels and milking utensils were compared using paired t-test. The statistically significant difference was observed between means of coliform count ( $P= 0.000$ ) from lactating camels and milking utensils (Table 11).

Table 11: Paired comparison of mean difference in total coliform count from lactating camels and milking utensils

| Sample types | Sample Size | *Mean of the differences | SD   | 95% CI       | T-test | df | P-value |
|--------------|-------------|--------------------------|------|--------------|--------|----|---------|
| Udder-MU     | 72          | -2.60                    | 1.19 | -3.00, -2.20 | -13.13 | 35 | 0.000   |

\*  $\text{Log}_{10} \text{CFU ml}^{-1}$  values, MU= milking utensils, SD= standard deviation of the mean difference, CI= confidence level of the mean difference, df = degree of freedom

#### 4.4. Qualitative analysis of milk for bacteria

Bacteriological examination was carried out on all of the composite udder milk samples and milk samples from milking utensils to isolate and identify the bacterial species present in camel milk. Accordingly different species of bacteria were isolated (Table 12). The Gram- positive cocci were dominant among the bacterial isolates from composite udder milk samples. The most frequently isolated bacteria were *Staphylococcus* species. The relative isolation of these bacteria was 26.30%, of which *Staphylococcus aureus* comprised of 13.80 %, *S. hyicus* 5.47%, *S. intermedius* 3.39% and *S. epidermidis* 3.65 %. Out of 5.99 % of *Streptococcus* species obtained, *Streptococcus agalactiae* was represented by 3.39 %, followed by *S. dysgalactiae* 1.04 % and *S. uberis* 1.56 %. In addition, the isolation of other bacteria such as *Micrococcus* spp., *Enterococcus faecalis*, *Rhodococcus equi*, *Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Mannheimia hemolytica*, *Pseudomonas* spp., and *Flavobacterium* spp. was 19.01 %, 3.65 %, 0.52 %, 2.06 %, 5.54 %, 8.33 %, 3.91 %, 2.08 %, 3.39 %, 2.86 %, and 0.26 %, respectively. *Staphylococcus* spp. was most frequently recovered



organism from milking utensils. *Staphylococcus epidermidis* was most frequently recovered (22.22 %) followed by *S. aureus* (11.11 %), *S. hyicus* (2.78 %) and *S. intermedius* (2.78 %). The other bacteria recovered from milk utensils comprised of *Micrococcus* spp. (5.56 %), *Streptococcus agalactiae* (5.56 %), *S. dysgalactiae* (2.78 %), *S. uberis* (2.78 %), *Enterococcus faecalis* (2.78 %), *Rhodococcus equi* (11.11 %), *Bacillus* spp. (8.33 %), *Corynebacterium* spp. (5.56 %), *Escherichia coli* (30.56 %), *Klebsiella pneumonia* (19.44 %), *Enterobacter aerogenes* (2.78 %), *Mannheimia hemolytica* (11.11 %), *Pseudomonas* spp. (16.67 %), and *Flavobacterium* spp. (8.33 %).

From the data shown on table 13, clinically mastitic udder have role as a source of *Staphylococcus aureus*, *S. hyicu*, *Streptococcus agalactiae*, *S. dysgalactia*, *Enterococcus faecalis*, *Bacillus* spp., *Escherichia coli* and *Mannheimia hemolytica* in camel milk. Subclinical mastitis acts as a source of contamination for microbes such as *Staphylococcus aureus*, *S. hyicus*, *S. intermedius*, *Enterobacter aerogenes*, *Escherichia coli* and *Corynebacterium* spp.. Likewise, milk form the also yielded several bacteria which included *S. aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. uberis*, *Enterococcus faecalis*, *Klebsiella pneumonia* and *Pseudomonas* spp.. On the other hand, milking utensils served as an important source of *Escherichia coli* and *Staphylococcus epidermidis*.



Table 12: Bacterial species recovered from camel milk samples

| Bacterial isolates              | Clinical<br>mastitis | Sub-clinical<br>mastitis | Healthy<br>udder | Total udder | Milking<br>utensils |
|---------------------------------|----------------------|--------------------------|------------------|-------------|---------------------|
| <i>Staphylococcus aureus</i>    | 5 (31.25 %)          | 36 (45.57 %)             | 12 (4.15 %)      | 53 (13.80%) | 4 (11.11%)          |
| <i>S. hyicus</i>                | 2 (12.50 %)          | 7 (8.86 %)               | 2 (0.69 %)       | 21 (5.47%)  | 1 (2.78%)           |
| <i>S. intermedius</i>           | 0 (0.00 %)           | 13 (3.39 %)              | 0 (0.00 %)       | 13 (3.39%)  | 1 (2.78%)           |
| <i>S. epidermidis</i>           | 0 (0.00 %)           | 0 (0.00 %)               | 14 (4.84 %)      | 14 (3.65 %) | 8 (22.22%)          |
| <i>Micrococcus</i> spp.         | 0 (0.00 %)           | 32 (40.51 %)             | 41 (14.2 %)      | 73 (19.01%) | 2 (5.56%)           |
| <i>Streptococcus agalactiae</i> | 1 (6.25 %)           | 3 (3.80 %)               | 9 (3.11 %)       | 13 (3.39 %) | 2 (5.56%)           |
| <i>S. dysgalactiae</i>          | 1 (6.25 %)           | 3 (3.80 %)               | 0 (0.00 %)       | 4 (1.04%)   | 1 (2.78%)           |
| <i>S. uberis</i>                | 0 (0.00 %)           | 2 (2.53 %)               | 4 (1.38 %)       | 6 (1.56%)   | 1 (2.78%)           |
| <i>Enterococcus faecalis</i>    | 1 (6.25 %)           | 6 (7.59 %)               | 7 (2.42 %)       | 14 (3.65 %) | 1 (2.78 %)          |
| <i>Rhodococcus equi</i>         | 0 (0.00 %)           | 0 (0.00 %)               | 2 (0.69 %)       | 2 (0.52%)   | 4 (11.11%)          |
| <i>Bacillus</i> spp.            | 1 (6.25 %)           | 5 (6.33 %)               | 4 (1.38 %)       | 10 (2.60%)  | 3 (8.33%)           |
| <i>Corynebacterium</i> spp.     | 0 (0.00 %)           | 16 (20.25 %)             | 0 (0.00 %)       | 16 (5.54 %) | 2 (5.56%)           |
| <i>Escherichia coli</i>         | 3 (18.75 %)          | 29 (36.71 %)             | 0 (0.00 %)       | 32 (8.33%)  | 11 (30.56%)         |
| <i>Klebsiella pneumonia</i>     | 0 (0.00 %)           | 5 (6.33 %)               | 10 (3.39 %)      | 15 (3.91%)  | 7 (19.44%)          |
| <i>Enterobacter aerogenes</i>   | 0 (0.00 %)           | 10 (12.66 %)             | 2 (0.69 %)       | 8 (2.08%)   | 1 (2.78%)           |
| <i>Mannheimia hemolytica</i>    | 2 (12.5 %)           | 4 (5.06 %)               | 7 (2.42 %)       | 13 (3.39%)  | 4 (11.11%)          |
| <i>Pseudomonas</i> spp.         | 0 (0.00 %)           | 0 (0.00 %)               | 11 (3.80 %)      | 11 (2.86 %) | 6 (16.67%)          |
| <i>Flavobacterim</i> spp.       | 0 (0.00%)            | 0 (0.00%)                | 1 (0.35 %)       | 1 (0.26 %)  | 3 (8.33 %)          |
| Total sample examined           | 16                   | 79                       | 289              | 384         | 36                  |



## 5. DISCUSSION

The results of the observational study indicated that pre-milking udder hygiene was not practiced in the study area. The urine of camel was used for washing of milking utensil and milker's hands due to scarcity of water in the area. Milking was practiced by using hands which were not generally cleaned. An individual camel-herder milked camels continuously without washing hands between milking of any two camels. These unhygienic practices could be responsible for the contamination of milk in the study area. Pre-milking udder preparation, has been studied to play an important part in the contamination of milk during milking (Galton *et al.*, 1986).

The finding of bacterial counts showed an increase in standard plate count and coliform counts in milking utensils than in lactating camels milk samples. This difference was statistically significant ( $P < 0.05$ ). The higher bacterial load in milking utensils could be attributed to contamination of milk outside the udder since there were no pre-milking udder preparation, milker's hand washing, and milking utensils washing by traditional camel herders in the study area. The increase in the coliform counts between the samples taken from camel udders and milking utensils might indicate coliform contamination due to unsanitary milking and milk handling practice with the possibility of milk contamination from feces of animals and/ or humans. Galton *et al.* (1986) mentioned that proper pre-milking udder preparations play an important part in the prevention of contamination of milk during milking, and unsanitary milking practices could result in elevated number of bacteria on the standard plate count. Environmental bacterial contamination of milk can be air-borne or depend on udder preparation, teat dipping and the cleaning procedures of milking equipment (Galton *et al.*, 1986).

The mean SPC in the present study was  $4.30 \log_{10}$  CFU/ml and  $5.34 \log_{10}$  CFU/ml for udder milk and milking utensils, respectively. The observation of the current investigation are consistent with Semereab and Molla (2001) who reported a mean SPC of camel milk samples from udder's and milking bowl's of  $4.30 \log_{10}$  CFU/ml and  $4.51 \log_{10}$  CFU/ml, respectively in Afar region. Likewise, Mengistu (2003) also described that the mean SPC of camel milk samples taken from udder and milking bowl's in Somali region were  $4.04 \log_{10}$  CFU/ml and  $4.74 \log_{10}$  CFU/ml,



respectively. The higher SPC in milk samples collected from milking utensils in this study might be attributed to the bacterial contamination as a result of unhygienic milking practices together with the use of camel urine for cleaning of milker's hand and milking utensils.

The mean coliform counts recorded in the current study were  $0.98 \log_{10}$  CFU/ml and  $3.57 \log_{10}$  CFU/ml for lactating camels and milking utensils, respectively. Compared to the present study, higher coliform counts of  $3.54 \log$  CFU/ml and  $3.82 \log_{10}$  CFU/ml of camel udder milk samples were recorded by Semereab and Molla (2001) and Mengistu (2003), respectively. The same investigators also reported higher coliform counts of  $3.84 \log_{10}$  CFU/ml and  $4.09 \log_{10}$  CFU/ml of milking bowel's milk samples, respectively. The lower coliform count in the current study could be attributed due to differences in relative proportion of coliforms in the three studies. All environmental components, which came in contact with milk are considered as potential sources of contamination of milk by coliforms, which, can be avoided by careful handling. The presence of coliform organisms in the milk indicates unsanitary condition of production. Their presence in large number in dairy products may pose a potential health hazards to consumer's health (Magessie and Fekadu, 1994).

It is pertinent to mention that no information was available on the limits of SPC and coliform counts for raw camel milk in Ethiopia. According to American and European community member states, the accepted limit for SPC and coliform counts for cow's raw milk are between  $5.30 \log$  CFU/ml and  $5.60 \log$  CFU/ml and  $2.02 \log$  CFU/ml, respectively (APHA, 1992; Heeschen, 1997). The results of the SPC in the present study were within the limits accepted in the USA and European Community member states for raw cow's milk. Nevertheless, the coliform count obtained was higher than the limits.

The prevalence of clinical mastitis as determined by clinical examination and culture was 16 (4.17 %). Abdurahman *et al.*, (1995) found that of the total 101 dromedary udders examined 6 (5.9 %) had clinical mastitis. Obied *et al.*, (1996) reported a prevalence rate of 19.5 % of clinical mastitis in camels. The CMT revealed a 20.57 % prevalence of sub-clinical mastitis. In eastern Ethiopia, Gizat and Bayleyen (2000), using CMT and culturing found 22.1 % prevalence of sub-clinical mastitis in camels. A relatively high level of sub-clinical mastitis was reported



elsewhere by different authors (Quandil and Ouadar, 1984; Abdurahman *et al.*, 1995; Obied *et al.*, 1996).

In the course of this study 18 bacterial species from raw milk samples were isolated. The most predominant bacteria isolated from milk samples obtained from lactating camels were *Staphylococcus aureus*, *S. hyicus*, *S. intermedius*, *S. epidermidis*, *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *Enterobacter aerogenes*, *Corynebacterium* spp., *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Klebsiella pneumonia* and *Mannheimia hemolytica*. Bacterial species recovered from the milking utensils, were also isolated from camel udder milk samples. This might not exclude the contribution of environmental and the role of milking utensils in bacterial contamination of camel milk since the bacterial isolates from the milking utensils could be found in the environment, on the exterior of the udder, even on the milker's hands and inside uncleaned milking utensils.

The infection of mammary gland may result in large numbers of bacteria in milk and may render milk unsuitable for human consumption (Adesiyun, 1994; Radostits *et al.*, 1994). Sub-clinical and clinical mastitis can contribute up to  $1.0 \times 10^4$  cfu/ml and  $1.9 \times 10^9$  cfu/ml of bacteria, respectively from the udder (Sumner, 1996). In this study, *S. aureus*, *S. hyicus*, *S. intermedius*, *S. epidermidis*, *S. uberis*, *S. dysgalactiae*, *S. agalactiae*, *Corynebacterium* spp., *Mannheimia hemolytica*, *Pseudomonas* spp. and *Micrococcus* spp. isolated from clinical and subclinical mastitis. *S. aureus*, *S. hyicus*, *S. intermedius*, *S. uberis*, *S. dysgalactiae*, *S. agalactiae*, *Enterococcus faecalis*, *E. coli*, *Mannheimia hemolytica*, *Bacillus* spp., *Pseudomonas* spp. and *Micrococcus* spp. are incriminated as causes of sub-clinical mastitis and clinical mastitis in the camel (Barbour *et al.*, 1985; Abdurahman *et al.*, 1995; Obied *et al.*, 1996; Gizat and Bayleyegn, 2000; Teketelew and Bayleyegn, 2001; Salah *et al.*, 2001). The contribution of mastitic udder in the bacteriological quality of camel milk is an established fact and therefore control of mastitis is one of the key elements in the production of high quality milk in addition to hygienic milking techniques. This is important from the food safety point of view.

In this context, Teshager and Bayleyegn (2001) indicated that several species of bacteria namely, *Streptococcus*, *Bacillus* spp., *Enterobacteriaceae* and *Pseudomonas aeruginosa* can act as



contaminants of raw camel milk. This happens when milk passes through the streak canal in healthy camels. This observation was in agreement with the present findings. In addition the isolation of coliform and *Bacillus* spp. from milking utensil milk sample indicated the probable contamination of milk from the unclean milker's hands, improperly cleaned milking utensils and use of camel urine for washing hands and milking utensils. The presence of coliform and *Bacillus* spp. in milk is undesirable as they may cause off flavors and reduction in the quality and shelf-life of milk (O'Connor, 1994).

There are a number of diseases such as brucellosis, listeriosis, tuberculosis, coxiellosis, yersiniosis, campylobacteriosis etc, transmissible to humans through the consumption of raw milk (Pal, 2007; Pal, 2012). Presently, the most important pathogenic bacteria which cause disease in humans are *Pathogenic E. coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Staphylococcus aureus* (Hahu, 1996; Heeschen, 1997; Pal, 2007). In the study areas, the camel milk was consumed in its raw form as boiling of camel milk was not practiced. The ingestion of raw milk poses a great public health concern (Pal, 2012). In current study, about 13.80 % and 11.11% of *Staphylococcus aureus* were isolated from milk samples of lactating camel and milking utensils, respectively. This may be major concern to human health since some strains of *Staphylococcus aureus* are capable of producing heat stable enterotoxins which can provoke food intoxication (vomiting and diarrhea) (Asperges, 1994; Pal, 2001). In the current study, 8.33% of milk samples from lactating camels, 30.56 % of samples from milking utensils contained *E. coli*. This may be associated with environmental contamination of milk after milking and mastitis. Although *E. coli* is a frequently occurring organism in milk and milk products, its role as a possible cause of disease is insufficient because *E. coli* normally is an ubiquitous organism (Hahu, 1996). However, the occurrence of pathogenic strains of *E. coli* such as O157:H7 in milk, could be hazardous for consumers. Therefore, identification of the strains of *E. coli* present in camel raw milk should be conducted.



## 6. CONCLUSION AND RECOMMENDATIONS

Camel is an important animal as its milk is the chief component of diet of camel herders in the study area. The difference between bacterial load and proportion of bacterial isolates of milk samples from the udder and milking utensils clearly demonstrated endogenous and exogenous sources of bacterial milk contamination. The occurrence of clinical and sub-clinical mastitis as well as high level of mastitis causing pathogens indicated the importance of mastitis as source of bacterial contamination. This contamination can easily result in the spoilage of milk. In the study area, camel milk is consumed in the raw state. Since raw milk serve as a vehicle for pathogenic microbes, the drinking of raw milk may pose a potential health hazard to the consumer. The present study suggested that camel milk was produced and handled under poor hygienic conditions posing a potential health risk to the consumers, and found to harbor varied type of bacterial spp.

Based on these findings, the following points are recommended :

1. The government of Ethiopia should try to settle the nomads so that they can get access to potable water and veterinary services for their animals, or arrange posts at certain intervals where such services could be availed.
2. Microbiological limits should be set on SPC and coliform counts of camel milk at national level in order to implement microbiological quality control of camel milk.
3. Hygienic practices should be promoted to the camel herders so as to milk, handle, store and transport camel milk under acceptable standards.
4. Proper utensils should be designed and promoted to camel herders.
5. Mastitis in camels should be controlled in order to produce good quality milk and to reduce public health risks associated with mastitic pathogens.
6. It is highly imperative to educate the public about the health hazards associated with the consumption of raw milk and milk must be properly boiled before ingestion to control the milk-borne zoonoses .



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## 8. ANNEXES

### Annex 1: Checklist used for recording observation study

#### 1. Milking techniques

- 1.1. Milking procedure used
- 1.2. Milking frequency per day
- 1.3. Milker's hand hygiene
- 1.4. Average number of camels milked by an individual
- 1.5. Washing of hands exercised between milking of any two camels
- 1.6. Milker's clothing

#### 2. Pre-milking udder preparation and hygiene

- 2.1. Flanks, udder and teats washed before milking

#### 3. Milking utensils

- 3.1. Source of water used for washing milking utensils
- 3.2. Frequency of cleaning of milking utensils

#### 4. Public health aspects

- 4.1. Habit of raw milk consumption
- 4.2. Presence of heat treatment of milk



## Annex 2: Grading and interpretation of the CMT

| symbol | meaning           | Visible reaction                                                                                                                                                                                                                                      | Interpretation                                        |
|--------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| T      | trace             | Mixture remain liquid                                                                                                                                                                                                                                 | 0-200,000 cell/ml<br>0-25% PMN                        |
| 1      | weak              | A slight slime forms and is seen to best advantage by tipping the paddle back and forth and observing the mixture as it flows over the bottom of the cup. Trace reactions tend to disappear with continued movement of the fluid                      | 150,000-500,000 cell/ml<br>30-40% PMN                 |
| 2      | Distinct positive | The mixture thickens immediately with gel formation. As the mixture is caused to swirl, it tends to move as a mass around the periphery of the cup exposed. When the motion is stopped the mixture levels out again and covers the bottom of the cup. | 800,000-5,000,000 cell/ml<br>60-70% PMN               |
| 3      | strong            | A gel is formed which causes the source of the mixture to become convex, viscosity is greatly increased so that there is a tendency for the mass to adhere to the bottom of the cup.                                                                  | Cell number generally over 5,000,000/ml<br>70-80% PMN |

PMN= polymorphonuclear leuckocytes

Source:- Schalm *et al* (1971).



### Annex 3: Primary identification of bacterial pathogens

|                                                               |                                                          |                                                                                  |                                                         |
|---------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------|
| One loopful of milk streaked on blood agar and MacConkey agar |                                                          |                                                                                  |                                                         |
| Incubation at 37°C for 24-48 hours                            |                                                          |                                                                                  |                                                         |
| Morphology characterization                                   |                                                          |                                                                                  |                                                         |
| Gram stain                                                    |                                                          |                                                                                  |                                                         |
| Gram positive                                                 |                                                          | Gram negative rod                                                                |                                                         |
| Cocci                                                         | Rods                                                     | Growth on<br>MacConkey agar                                                      | No growth on<br>MacConkey agar                          |
| Oxidative, cat+<br>= <i>Micrococcus</i>                       | Fermentative,<br>cat+/oxidase- =<br><i>Coryn.spp</i>     | oxidative,<br>cat+/oxidase+=<br><i>Pseudo. Spp</i>                               | Oxidative, cat+/<br>oxidase +=<br><i>Flavobacterium</i> |
| Fermentative, cat+<br>= <i>Staphylococcus</i>                 | Fermentative,<br>cat-/oxidase-<br>= <i>Act. Pyogenes</i> | Fermentative,<br>cat+/oxidase-<br><i>Past. Hemolytica</i>                        | Fermentative,<br>cat+/oxidase-<br><i>Past.spp.</i>      |
| Fermentative, cat+<br>= <i>Streptococcus</i>                  | Unreactive,<br>cat+/oxidase-<br>= <i>Rhodococcus</i>     | Fermentative,<br>cat+, oxidase-<br>= <i>Enterobacteriaceae</i>                   |                                                         |
| Unreactive, cat+<br>= <i>Rhodococcus</i>                      |                                                          | Unreactive,<br>cat+/oxidase+<br>= <i>Alcaligenes spp.</i><br><i>Past. cabali</i> |                                                         |

Source: Quinn, *et al.*(1994).



Annex 4: - Differential tests used for identification of coagulase positive and coagulase negative staphylococci.

| Tests                 | <i>S. aureus</i> | <i>S. intermedius</i> | <i>S. hyicus</i> | <i>S. epidermidis</i> | <i>S. lentus</i> | <i>S. chromogenes</i> |
|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|-----------------------|
| Coagulase             | +                | +                     | +                | -                     | -                | -                     |
| Hemolysis             | +                | +                     | -                | D                     | -                | -                     |
| Pigment production    | +                | -                     | -                | -                     | D                | +                     |
| Urease                | NT               | NT                    | NT               | +                     | -                | +                     |
| Novobiocin (5µg)      | NT               | NT                    | T                | S                     | R                | S                     |
| Polymyxin B(300 unit) | R                | S                     | R                | R                     | S                | S                     |

d= 11-89% positive; S= Susceptible, B= Zone of less than 10 mm); NT= not tested

Source: Quinn *et al.* (1994).

Annex 5: Differential tests used for identification of *Streptococcus* species

| Tests                                | <i>S. agalactiae</i> | <i>S. dysgalactiae</i> | <i>S. uberis</i> | <i>Ent. faecalis</i> |
|--------------------------------------|----------------------|------------------------|------------------|----------------------|
| CAMP test                            | +                    | -                      | -                | -                    |
| Aesulin hydrolysis on Edwards medium | -                    | -                      | +                | +                    |
| Colonies on Edwards M.               | Whitish              | Greenish               | Dark to brown    | Dark to brown        |
| Growth on MacConkey agar             | -                    | -                      | -                | +                    |

Ent.= *Enterococcus*

Source : Quinn *et al.* (1994)



Annex 6: Differential tests used for identification of *Actinomyces* and *Corynebacterium* species

| Test                    | <i>A. pyogenes</i>           | <i>C. bovis</i>                 | <i>C. ulcerans</i>                  |
|-------------------------|------------------------------|---------------------------------|-------------------------------------|
| Colonies on blood agar  | Pin point<br>hemolysis       | Small granular<br>Non hemolytic | Small translucent<br>Non haemolytic |
| Gram's reaction         | Gram positive,<br>small rods | Gram positive,<br>small rods    | Gram positive, small rods           |
| Catalase production     | -                            | +                               | +                                   |
| Growth on 9% NaCl       | -                            | +                               | -                                   |
| Fermentation of lactose | +                            | -                               | -                                   |
| Trehalose               | -                            | -                               | +                                   |

A= *Actinomyces*, C= *Corynebacterium*

Source: Carter. (1984)



# Annex 7: Differential tests used for identification of Enterobacteriaceae

| Tests                    | <i>E. coli</i>           | <i>K. pneumoniae</i>      | <i>Ent. Agglomerans</i>      | <i>C. freundii</i>           |
|--------------------------|--------------------------|---------------------------|------------------------------|------------------------------|
| Growth on MacConkey agar | Pink dry Colony          | Pink yellow mucoid colony | Pink large dry mucoid colony | Pink large dry mucoid colony |
| Gram's reaction          | Gram negative small rods | Gram negative small rods  | Gram negative small rods     | Gram negative small rods     |
| IMVIC                    |                          |                           |                              |                              |
| Indol                    | +                        | -                         | -                            | -                            |
| Methyl-red               | +                        | (-)                       | d                            | +                            |
| Voges proskauer          | -                        | +                         | d                            | -                            |
| Citrate utilization      | -                        | +                         | d                            | +                            |
| H <sub>2</sub> S         | -                        | -                         | -                            | (+)                          |
| Urease                   | -                        | +                         | (-)                          | D                            |
| Lysine decarboxylase     | (+)                      | +                         | -                            | -                            |

E= *Escherchia*, K= *Klebsiella*, C= *Citrobacter* , Ent= *Enterobacter* (+)= 76-89% of strain positive, (-)= 11-25% of strain positive, d= 26-75% of strain positive.

Source: Carter. (1984).

## Annex 8: Media used for isolation and counts of bacteria

**Blood agar base**, 500 g (Merck, Germany):

Composition (g/l): Nutrient substrate (heart extract and peptone) 20.0; sodium chloride 5.0; agar-agar 15.0. pH 6.8±0.2 at 25<sup>0</sup> c.



Preparations: Suspended 40 g in 1 litre of demineralised water by heating in a boiling water bath or in current of steam and autoclaved at  $121^{\circ}\text{C}$  for 15 minutes. Cooled to  $45\text{--}50^{\circ}\text{C}$ , added 5-8 % sterilized defibrinated sheep blood and mixed taking care to avoid bubble formation. Poured into Petri dishes.

**MacConkey**, 500 g (Merck, Germany):

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5. pH  $7.1\pm 0.2$ .

Preparation: Suspended 50 g in 1 litre of demineralized water by heating in boiling water bath in a current of steam and autoclaved for 15 minutes at  $121^{\circ}\text{C}$ .

**Edwards medium** (modified) 500 g (Oxoid, England):

Composition (g/l) : Lab-Lemco powder 10.0; peptone 10.0; asculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallous sulphate 0.3; agar 15.0. pH  $7.4\pm 0.2$ .

Preparation: Suspended 41 g in litre of distilled water and boiled to dissolve completely. Sterilized by autoclaving at  $115^{\circ}\text{C}$  for 20 minutes. Cooled to  $50^{\circ}\text{C}$ , added 5-7% of sterile bovine or sheep blood, mixed well and poured into plates.

**Nutrient agar**, 500 g (Oxoid, England):

Composition (g/l): 'Lab-Lemco' powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0. pH  $7.4\pm 0.2$ .

Preparation: Suspended 28 g in 1 litre of distilled water and boiled to dissolve completely. Sterilized by autoclaving at  $121^{\circ}\text{C}$  for 15 minutes.



**Trypton water**, 500 gm (Merck Darmstadt, Germany);

Composition (g/l): Peptone from casein 10.0; sodiumchloride 5.0. pH  $7.0 \pm 0.2$ .

Preparation: Dissolved 15 gm/l, autoclaved in 15 min at  $121^{\circ}\text{C}$ .

Procedure and interpretation: One millilitre of ether was added to a 5 ml portion of a 48 hrs culture growth at  $37^{\circ}\text{C}$  in a peptone water, shaken well and allowed to stand until the ether raised to the top. Then, Kovac's reagent was added gently to the side of the test tube and the formation of brilliant red ring between the medium and ether was indicative of an indole production.

**MR-VP broth** (Methyl Red- Voges-prouskauer) (500 g), (Merck, Germany):

Composition (g/l): Peptone from meat 7.0; D (+) glucose 5.0; tampon phosphate 5.0. pH  $6.9 \pm 0.2$ .

Preparation: Suspended 17 g in litre of demineralised water; dispensed 5 ml portion into tubes and autoclaved 15 minutes at  $121^{\circ}\text{C}$ .

Test procedures and interpretation:

Methyl Red test: The test organism was inoculated into 5 ml MR-VP broth media and then incubated for 48 hrs at  $37^{\circ}\text{C}$ . After incubation, 5 drops of methyl red solution was added and a positive reaction indicated by a distinct red colour indicating acidity (PH= 4.4-6.0) and a negative reaction indicated by orange to yellow colour.

Voges – Proskauer test : The test organism was inoculated into 1 ml MR-VP broth media and then kept for 48 hrs at  $37^{\circ}\text{C}$  . After inoculation, 0.6 ml of 5% alphanaphtol solution was added, and then 0.2 ml of 40% KOH containing 0.3% creatine was added. The solution was shaken well and left for 5-10 minutes. A positive reaction indicated by development of bright pink or eosin



red colour indicating acidity (PH = 4.4 to 6.0) and a negative reaction indicated by no colour change.

**Simmon's citrate agar, 500 ( DCM, USA):**

Composition (g/l): Ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.0; sodium chloride 5.0; sodium citrate 2.0; magnesium sulphate 0.2; agar 15.0 and bromothymol blue 0.08. pH  $6.9 \pm 0.2$

Preparation: Dissolved 22.5 g/l, autoclaved at  $121^{\circ}\text{C}$ , prepared slant agar tube.

Inoculation: Slants were inoculated with isolates picked from several similar colonies. Incubated at  $37^{\circ}\text{C}$  for 24 to 48 hours.

Interpretation: Any blue colour is an indication of citrate utilization.

**O-F basal medium ( Oxidation – Fermentation), 500 g (Merck, Germany):**

Composition ( g/l): peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; dipotassium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5. pH  $7.1 \pm 0.2$ .

Preparation: Suspended 11 g in 1 litre of demineralized water by heating in a boiling water bath or in a current of steam: autoclaved 15 minutes at  $121^{\circ}\text{C}$ ; at approximately  $50^{\circ}\text{C}$  mix in 100 ml/l of a filter sterilized 10% solution of D (+) glucose; lactose or other carbohydrate; dispensed into tubes to give depth of approximately 5 cm, in half of the tubes immediately overlaid the medium with a 1 cm layer of sterile paraffin viscous.

Procedure and evaluation: Inoculated one tube with and without paraffin seal with a pure culture of the microorganism to be examined down to the bottom of the tube by stabbing techniques. The organism used for inoculation should be in the logarithmic phase of growth. Incubation was done at least 48 hours at the optimal temperature

Interpretation: A yellow colouration in both the open and paraffin sealed tubes signifies fermentative degradation whereas yellow colouration of the open tubes alone indicates that the carbohydrate in question is broken down by oxidation and absence of colour change indicates unreactive



**Phenol red broth base, 500 g (Merck, Germany)**

Composition (g/l): phosphate from casein 5.0; peptone from meat 0.5; sodium chloride 5.0; phenol red 0.018. pH  $7.4 \pm 0.2$ .

Preparation: Suspended 15 g in 1 litre of demineralised water; dispensed into tubes. If necessary, autoclaved 15 minutes at  $121^{\circ}\text{C}$ ; at less than  $60^{\circ}\text{C}$  added the reactants (final concentrations 5-10 g/l) as sterile solution.

Test for enzymes:

**Catalase Test:** This test demonstrate the presence of catalase, an enzyme that catalase the release of oxygen from hydrogen peroxide. A drop of 3% hydrogen peroxide was poured on the glass slide and then small amount of the culture to be tested was picked from a nutrient agar with a clean sterile platinum loop or a clean, thin glass rod and this was added into hydrogen peroxide solution held on glass slide. The production of gas bubbles indicated a positive reaction. It occurred almost immediately. A false positive reaction may be obtained if the culture medium contains catalase (ex. blood agar) or if an iron wire loop is used (Collee, 1989).

**Oxidase test:** This test depends on the presence of oxidase in the bacteria that will catalyse the transport of electrons donors in the bacteria and redox dye tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour. The dye is used for screening species of *Alcaligenes*, *Pseudomonas*, *Flavobacterium* and *Pasteurella spp.* which give positive reactions and excluding the Enterobacteriaceae, all species of which give negative reactions.

**Urease test.** 500g (Merck, Germany): Composition (g/l): Yeast extract 0.1; potassium dihydrogen phosphate 9.1; disodium hydrogen sulphate 9.5; urea 20.0; phenol red 0.01.

Preparation: Dissolved 38.5 g/l and sterilize in 5 minutes in a current of steam under mild condition. Dispensed approximately 3 ml into test tubes.

Experimental procedure and evaluation: Inoculated the medium massively with pure culture under investigation. The inoculated medium was incubated at  $37^{\circ}\text{C}$  for 48 hrs.

Interpretation: Red= urea positive; Yellow= urea negative



**Plate count agar** (for standard plate count), 500 g (OXOID, England):

Composition (g/l): peptone from casein 5.0; yeast extract 2.5; D (+) glucose 1.0; agar-agar 14.0.

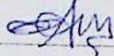
Preparation: Dissolved 22.5 g/l and sterilized by autoclaving at 115° c for 20 minutes.



## 9. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name Akulu Feleke

Signature 

Date of submission \_\_\_\_\_

This thesis has been submitted for examination with my approval as University advisor.

Dr. Tesfay sisay (DVM, MSc, PhD; Associate Professor) \_\_\_\_\_

Prof. M. Pal (PhD, Professor) \_\_\_\_\_

