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CROSS-SECTIONAL STUDY OF MASTITIS IN CAMELS (CAMELUS DROMEDARIUS) IN SELECTED SITES OF ETHIOPIA

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A thesis submitted in partial fulfillment for the degree of Master of Science in Tropical Veterinary Epidemiology at Freie Universität Berlin and Addis Ababa University

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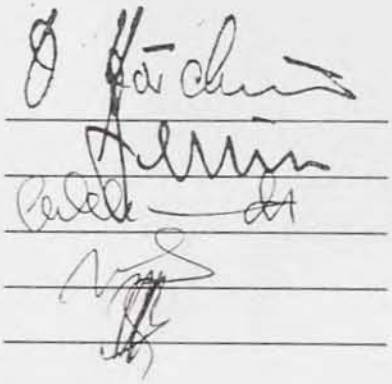

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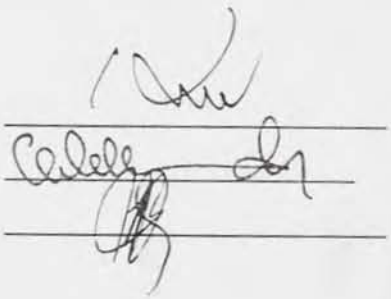

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
ANRS	Afar National Regional State
ATP	Adenosine Triphosphate
cm	Centimeter
CMT	California Mastitis Test
CNS	Coagulase-negative Staphylococci
DMSCC	Direct Microscopic Somatic Cell Count
FAO	Food and Agriculture Organization
FUB	Free University of Berlin
GM	Gram
ILCA	International Livestock Center for Africa
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate tests
Km	Kilometer
Log SCC	Logarithm of Somatic Cell Count
MAP	Major Pathogen
MF	Magnification Factor
MIP	Minor Pathogen
m	Meter
ml	Milliliter
mm	Millimeter
µg	Milligram
NaCl	Sodium Chloride
NCCLS	National Committee for Clinical Laboratory Standards
NI	Non-Infected
No.	Number
O-F	Oxidative-Fermentative
SCC	Somatic Cell Count
T	Trace
TSI	Triple Sugar Iron Agar
U	Units
+ve	Positive
-ve	Negative

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ABSTRACT

One thousand and twelve udder quarters from 253 traditionally managed lactating camels (*Camelus dromedarius*) were investigated according to multi-stage sampling in the study sites. Out of the 1012 udder quarters, 35 (3.46%) quarters were blind and 21 (2.08%) quarter milk samples were lost during handling. The remaining 956 quarter milk samples (466, 305, 185 from Negele (Borena Region), Dire Dawa, and Gewane (Afar Region), respectively) were tested for California Mastitis Test (CMT), Somatic Cell Counts (SCC), and bacteriological analysis.

The prevalence of clinical mastitis was determined based on the inspection of the udder and milk, while prevalence of sub-clinical mastitis was calculated based on the results of bacteriological examinations (major pathogens). High prevalence of sub-clinical mastitis was recorded from quarter milk samples in Dire Dawa (46.2%, n = 141) followed by Gewane (Afar Region) (45.4%, n = 84) and Negele (Borena Region) (37.1%, n = 173). Clinical mastitis was observed only in five (1.1%) quarters in Negele (Borena Region).

A total of 956 quarter milk samples from 253 traditionally managed lactating camels were collected aseptically and examined using standard microbiological techniques irrespective of the results of CMT and SCC. Five hundred and seventy one (59.73%) quarter milk samples had microorganisms and 385 (40.27%) quarter milk samples were found negative for microbial analysis. Out of 571, 428 (75.00%) isolates were identified as major pathogens (MAP) and 143 (25.00%) isolates were considered minor pathogens (MIP). The common pathogens isolated from sub-clinical mastitis were *S. aureus* and *E. coli* accounting for 24.7% (n = 139) and 17.4% (n = 98) of the total isolates of sub-clinical mastitis. The microorganisms isolated from clinical cases were *S. aureus* 22.2% (n = 2), *S. hyicus* 22.2% (n = 2), *Streptococcus uberis* 22.2% (n = 2), *Bacillus cereus* 11.1% (n = 1), *E. coli* 11.1% (n = 1), and *S. epidermidis* 11.1% (n = 1).

For the analysis, CMT scores of negative (0) and trace (±) were considered as negative and different intensities of CMT positive (1, 2, 3) were considered as positive. A positive correlation was found between CMT scores and bacteriological groupings (MAP, MIP, and NI) (χ^2 = 49.98, *P*-value = 0.00). Out of 933 quarter milk samples examined, 133 (14.26%) were CMT positive. The CMT

positive cases were associated with quarter milk samples that had both major pathogens (MAP) and minor pathogens (MIP) (81 (60.90 %) and 37 (27.82%), respectively). However, 15 (11.28%) CMT positive cases were from non-infected (NI) quarter milk samples.

Use of both logSCC and SCC cut-off level 2.5 x 10^5 ml^{-1} indicated strong correlation (*P*-value = 0.00) with CMT scores. But the differences among the medians log SCC of bacteriological groupings (major pathogens (MAP), minor pathogens (MIP), and non-infected (NI)) were not significant (Kruskall Wallis = 2.868, *P*-value = 0.238). The application of the cut-off level of 2.5 x 10^5 ml^{-1} also indicated less agreement for bacteriological groupings ($\chi^2 = 2.29$, *P*-value = 0.319).

Results of risk factors analysis showed that the use of anti-suckling devices can be considered as contribution factor ($\chi^2 = 7.77$; *P*-value = 0.005) in spreading of intra-mammary infection in camels. In our study, other factors such as tick infestations, teat lesions, conformation of the udder, previous history of mastitis, stage of lactation, parity, and age were found to be less important to the occurrence of camel mastitis in the study sites as compared to use of anti-suckling devices in camels.

In conclusion, the results of the present study revealed that mastitis in camel is prevalent in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region). California Mastitis Test (CMT) can be applied for the detection of the udder infection in camels. The pathogenic bacteria that cause mastitis in camels are similar to those of the cows. Use of anti-suckling devices is responsible for spreading of intra-mammary infection in camels.

1. INTRODUCTION AND OBJECTIVES

There are two types of camels in the world: the one-humped camel (*Camelus dromedarius*) and the bactrian two-humped camel (*Camelus bactrianus*). The bactrian camel is mainly found in the regions of the European-Russian continent. It accounts for 5% of the world camel population. The dromedary camel is the most dominant and widely distributed in the tropics and sub-tropics of the African and Asian continents. It is numerous in the arid and semi-arid areas of Africa, particularly in the arid and semi-arid lowlands of eastern Africa. The population in this region is 11 million which accounts for 78% of the Africa and 57% of the camel population in the world (FAO, 1997).

According to FAO (1992), consumption of livestock products increased 2-3 times more in developing countries as compared to developed countries. This is attributed to the high population growth and an increasing urbanization in the third world. The FAO report indicated that the food supply must be improved, both in quantity and quality. It is well known that camels play major roles in improving the socio-economic status and survival of the desert dwellers and are major sources of protein and energy for them. This is due to a number of specific anatomical and physiological characteristics of the camel as well as low susceptibility to diseases (Schwartz and Dioli, 1992).

Schwartz and Dioli (1992) recorded that the camel milk contains the necessary proteins, sugars, fats, minerals and vitamins for the young calves and is valuable food for the people. Beside that camel milk is a rich source of vitamin C for the desert people who are unable to get it from other sources (Schwartz and Dioli, 1992; Wilson, 1998). On the other hand udder infection was considered as one of the main constraints for camel rearing. For instance, it has been noticed in the slaughter houses that early culling of female camel in Iraq is attributed to chronic mastitis and infertility (Al-Ani and Al Shareefi, 1997). During the last decades the disease has been reported from a number of camel-rearing countries in Africa such as Egypt (Moustafa *et al.*, 1987; Karmy, 1990), Somalia (Arush *et al.*, 1984; Abdurahman *et al.*, 1991), Sudan (Abdurahman *et al.*, 1995; Obied *et al.*, 1996) and Kenya (Younan *et al.*, 2001).

In Ethiopia, camels are kept in arid and semi-arid lowlands of Borena, Ogaden, and Afar regions. The camel population is estimated at one million in the country (Teka, 1991). The same author has also reported that the camel milk is the main food for the nomads in pastoral production system of Ethiopia, and they consumed it in raw state. Based on that, Almaw and Molla (2000), Salah (2000), and Bekele and Molla (2001) investigated the udder infections in the dromedary camels in eastern, south western, and north eastern parts of the country. They also recommended that an extensive research is needed on the epidemiology and etiology of the camel mastitis as well as the diagnostic tools and related factors, which influence the prevalence of the disease. Therefore the aims of the study were:

- To determine the prevalence of clinical and sub-clinical mastitis in the camel in Dire Dawa, Gewane (Afar Region), and Negele (Borena Region) of Ethiopia.

- To evaluate California Mastitis Test (CMT) and Somatic Cell Counts (SCC) based on microbiological examinations for the detection of camel mastitis.

- To analyze some of the risk factors that affects the occurrence of camel mastitis.

- To estimate the susceptibility levels of bacteria isolated from camel mastitis to commonly used anti-microbials.

2.LITERATURE REVIEW

2.1. Distribution and utilization of the camel

The dromedary or one-humped camel (*Camelus dromedarius*) is the one of two species within the genus *Camelus*, the other being the bactrian or two-humped camel (*Camelus bactrianus*). The dromedary is originally a tropical animal. However, some dromedary keeping countries are located outside the tropics. It has been supposed that the dromedary was first domesticated in southern Arabia, by people who had no other domestic animals for milk production, and then spread to north Africa. These animals were also found in north and south America, western Asia and India, Canary Islands, Caribbean, Italy and southern Spain (Knoess, 1977).

In Africa camels produce milk, meat, blood, hair, and hide. They are also used for riding, draught purposes, and as pack animals (Knoess, 1977; Schwartz and Dioli, 1992).

2.2. Adaptation and feeding of the camel

The camels are well adapted to the arid and extremely arid zones. This is due to the fact that the camels can change their body temperature to as low as 34°C and as high as 41°C and produce small amounts of urine which is highly concentrated. Water conservation by means of physiological mechanisms alone is not, however, the only adaptive feature of the camel which also possesses behavioral and anatomical adaptation as well as additional physiological ones (Wilson, 1998). The camels have excellent feed conversion efficiencies such that they convert the scattered vegetation of the desert into highly nutritious food and other forms of energy. The camels can browse, eat bushes and branches of trees or graze on grass. They can be fed like cattle and will eat straw, hay, silage, grains and cakes. They can eat sharp, thorny plants, which other animals can not eat. In dry seasons, when feed becomes scarce the camels can live on hump fat and survive in this way for a very long period. A further advantage is the low susceptibility of the camels to diseases (Schwartz and Dioli, 1992; Wilson, 1998).

2.3. Pastoralist areas in Ethiopia

The pastoralist areas of Ethiopia are located in the lowlands of the country and cover 48% (590,000 Km^2). The lowland composes about one million camels corresponding 12% of the total livestock population in the lowlands. The major pastoralist groups are Oromo, Afar, and Somali (Teka, 1991; Coppock, 1994). According to the classification of Coppock (1994) the pastoralist areas in Ethiopia cover the arid, semi-arid, and sub-humid zones.

2. 3.1. Economic potential and major constraints for camel rearing

Most camels are kept by nomadic pastoralists, and play an essential role in their subsistence economy. The economic values of keeping camels are specifically for milk and meat production. In addition, they serve as an investments, provide employment and cash income generation. Only in recent years there have been attempts to market camel milk. Camel milk is not readily sold by owners in the pastoral production system of the east Africa because of socio-cultural restraints. However, camel milk is now being marketed in an industrial manner in Saudi Arabia, and on a smaller scale in Mauritania, Somalia, and in a limited scale in the northern Kenya. Its also started to be sold as a health food in Europe (Schwartz and Dioli, 1992; Köhler-Rollefson, 1993).

The major constraints of camel production in arid and semi-arid areas are shortage of feed and water, camel diseases such as camel trypanosomiasis, respiratory diseases, helminthosis, tick infestation and sarcoptic mange, low production rate and high mortality in calves and abortion. Infrastructure and marketing of camels as well as the products and by products are underdeveloped (Schwartz and Dioli, 1992; Wilson, 1998).

2.4. Milk production

Camel milk is one of the most valuable food resources in arid and semi-arid of eastern Africa. It contains all the essential nutrients. The total dry matter ranges from 12 to 15%, protein from 2.7 to 4.5%, fat from 2.9 to 5.2% and lactose up to 5.5%. Camel milk is a rich source of vitamin C (may reach 2.9 mg/100 g). This is very important for desert people who are unable to get this vitamin from fruits and vegetables. Lactation lengths likewise show large variations of 8 months

to almost 2 years with estimated milk yields of 800 to over 4000 Kg per lactation. Recent research on the chemistry and biochemistry of camel milk and advances in processing technology have shown that it is possible to produce both butter and cheese without too much difficulty. Other traditional products such as fermented milk are not difficult to make it and are highly appreciated by the owners (Schwartz and Dioli, 1992; Wilson, 1998).

2.4.1. Milking and related problems

The camel udder has four quarters, one teat per quarter and two streak canals per teat. The teats can be well formed like those of cows but usually are not as long or as thick. They resemble those of heifers. Under traditional management, where most milk is produced, men mainly milk camels. Milking frequencies are variable and depend on the needs of the owners, climate and feed conditions. Generally, higher frequencies produce high total milk yield, which is noticeable up to four times per day. At the early stage of lactation, the calf has to suckle first to stimulate milk let-down, after the udder has been stimulated the let-down is only for short duration. Hence, the milking must be done as quickly as possible. Aggressiveness towards the calf or milker by lactating camels is common (Schwartz and Dioli, 1992; Wilson, 1998).

2.5. Udder infection

Mastitis is a complex disease occurring worldwide among dairy animals, with heavy economic losses largely due to sub-clinical mastitis. There is extensive literature on bovine mastitis and to some extent on ovine and caprine mastitis. Very little is known about mastitis in camel. The disease was believed to be rare in the camel. However, cases of mastitis in the camel have been reported from different countries, e.g. Egypt (Moustafa *et al.*, 1987; Karmy, 1990); India (Kapur *et al.*, 1982); Saudi Arabia (Barbour *et al.*, 1985; Hafez *et al.*, 1987; Ramadan *et al.*, 1987); Iraq (Al-Ani and Al Shareefi, 1997); Somalia (Arush *et al.*, 1984; Abdurahman *et al.*, 1991); Sudan (Abdurahman *et al.*, 1995; Obied *et al.*, 1996); United Arab Emirates (Quandil and Ouadar, 1984), Ethiopia (Almaw and Molla, 2000; Salah, 2000; Bekele and Molla, 2001), and Kenya (Younan *et al.*, 2001).

2.5.1. Pathogens causing camel mastitis

Camels under traditional management system are usually kept far from urban. Thus, bacteriological examination of the camel milk was considered difficult (Abdurahman *et al.*,1995). *Streptococcus agalactiae* and *Staphylococcus aureus* were the main causes of camel mastitis (Obied *et al.*, 1996). Recently, Almaw and Molla (2000) and Bekele and Molla (2001) have isolated other *Streptococcus spp.* and coagulase-negative Staphylococci from mastitic milk of dromedary camel. Barbour *et al.* (1985) and Al-Ani and Al Shareefi (1997) considered *Micrococcus spp.* as important causative agents of camel mastitis.

E. coli has been reported as major pathogen causing mastitis in the camel (Saad and Thabet, 1993; Abdurahman *et al.* 1995; Bekele and Molla, 2001). *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* have been isolated from both clinical and sub-clinical camel mastitis (Kapur *et al.*, 1982; Quandil and Ouadar, 1984; El-Jakee, 1998; Bekele and Molla, 2001). On the other hand, *Proteus vulgaris* and *Aerobacter spp.* have been isolated from normal camel udder tissue and milk, respectively (Obied *et al.*, 1996; El-Jakee, 1998).

Relationship between occurrence of camel mastitis and presence of *Corynebacterium spp.*, *Actinomyces spp.*, and *Bacillus spp.* were reported in Iraq (Al-Ani and Al Shareefi, 1997), Egypt (El-Jakee, 1998), and Ethiopia (Almaw and Molla, 2000; Bekele and Molla, 2001). Camel mastitis caused by anaerobic *Clostridium perfringens* was considered as a problem in Egypt (El-Jakee, 1998). *C. albicans* has also been reported as a causative agent of camel mastitis (Quandil and Ouadar, 1984; El-Jakee, 1998).

2.5.2. Risk factors of camel mastitis

The use of devices to prevent the calves from sucking their dams, together with heavy tick infestation of the udders and the treatment of infected quarters by cauterization have been considered as predisposing factors for camel mastitis (Abdurahman *et al.*, 1995; Obied *et al.*, 1996). Furthermore, Bekele and Molla (2001) considered that the heavy tick infestation, use of anti-suckling devices, and the thorny bushes in pastoral areas might be responsible for udder abnormalities and deformities and blind teats in Afar milking camels. They also reported a positive correlation between trauma and the occurrence of camel mastitis. Recently, Younan *et*

al. (2001) reported that the camel pox might be considered as a contributing factor in the spreading of intra-mammary infection caused by *Streptococcus agalactiae* in Kenya.

2.5.3. Forms of camel mastitis

The forms of mastitis in camel can be clinical mastitis (including peracute, acute, and chronic mastitis) or sub-clinical mastitis. Clinical mastitis in camel is characterized by hardening and swelling of the udder, pain on palpation and visible alteration of the colour and consistency of milk. Thus, clinical mastitis can be detected by examination of the udder and/ or of the milk (Obied *et al.*, 1996).

The term peracute mastitis refers to severe inflammation, with swelling, heat and pain of quarter, with a marked systemic reaction that may be fatal. The change in the mammary glands in acute mastitis is similar to those of peracute but the systemic signs are less severe (Quinn *et al.*, 1994; Radostits *et al.*, 2000). In India, Kapur *et al.* (1982) recorded a case of peracute mastitis caused by *Klebsiella spp.* and *E. coli* that resulted from contamination during udder surgery. While Quandil and Ouadar (1984) reported acute form of camel mastitis.

In Saudi Arabia, Ramadan *et al.* (1987) described unilateral chronic mastitis caused by *S. aureus* and *Pasteurella haemolytica* in three lactating camels due to obstruction of the teat canals by keratin. He indicated that the camels with previous history of acute bacterial mastitis lead to chronic mastitis with subsequent fibrosis and keratinization of the udder tissue. Chronic cases of the camel mastitis due to *S. aureus* have also been reported in Iraq (Al-Ani and Al-Shareefi, 1997).

Sub-clinical mastitis was reported in Sudan (Obied *et al.*, 1996; Abdurahman *et al.*, 1995) and Ethiopia (Almaw and Molla, 2000; Bekele and Molla, 2001). The same authors indicated that the term sub-clinical mastitis in camels refers to the existence of inflammation in the absence of gross sign and can be detected by indirect tests such as California Mastitis Test (CMT) and Somatic Cell Count (SCC) as well as microbiological examinations.

2.5.4. Diagnosis of camel mastitis

Clinical mastitis can be detected by examination of the udder and/ or of the milk as well as using indirect tests (Obied *et al.*, 1996). Bacteriological examinations of the milk, California Mastitis Test (CMT), and Somatic Cell Counts (SCC) have been used as diagnostic tools to detect sub-clinical mastitis in camels (Obied *et al.*, 1996; Abdurahman *et al.*, 1995; Almaw and Molla, 2000; Bekele and Molla, 2001).

The value of the CMT for the early detection of mastitis in camel herds was indicated by Moustafa *et al.* (1987). A study on the sensitivity and specificity of CMT in Kenya (Younan *et al.*, 2001) has shown that the CMT can be applied as a screening test for the detection of intra-mammary infection with *S. aureus* and *Streptococcus agalactiae* in camels. This result supports that of Bekele and Molla (2001) who reported during their study on camel mastitis a positive correlation between CMT scores and bacteriological results. Moreover, Obied *et al.* (1996) found a strong correlation between leukocyte counts of the camel milk and the CMT scores. An increase of SCC with the degree of CMT and their value in predicting the infection status of the udder in lactating camels has also been reported by Abdurahman (1996) and Al-Ani and Al-Shareef (1997).

Variations in the leukocyte counts of colostrum, normal milk, and dry-period milk from camels have been reported by Moustafa *et al.* (1987). There were increases in the Somatic Cell Counts (SCC) in camel milk with age, stage and number of lactations, and the results of the rapid mastitis test (CMT) were directly correlated with the leucocyte counts (Kospakov, 1976). However, Obied *et al.* (1996) found no relationship between leukocyte counts and different stages of the same lactation in camels. They considered an increase in the somatic cell counts over 500,000 to be an indication of the udder infection in camels. Abdurahman (1996) in the study of sub-clinical mastitis in seven lactating bactrian camels (*Camelus bactrianus*) kept at a zoological park in Sweden, showed that camels free of udder infection had a higher basal SCC than cows. The author also recorded that the cell fragments may be counted as cells in microscopic counting. Abdurahman (1992) previously recorded the presence of large numbers of the nucleated cell fragments in the camel milk. Similar cell fragments in goat milk did not react with CMT reagents (Schalm *et al.*, 1971).

Some other diagnostic tools have been used for the detection of camel mastitis. Saad and Thabet (1993) have reported a strong correlation between Whiteside test and bacteriological results of camel milk samples. Abdurahman *et al.* (1995) indicated that milk samples from quarters infected with major pathogens had higher values for CMT, SCC and Adenosine Triphosphate (ATP) than samples from quarters that were infected with minor pathogens and from not infected quarters. The NAGase test has been used for predicting of udder infection in cow. The test is based on the measurement of a cell-associated enzyme (N-acetyl-β-D-glucosaminidase) in the milk. A high level of the enzyme indicates a high cell counts (Radostits *et al.*, 2000). In Sudan, Abdurahman (1995) measured the NAGase activity and concentration of serum albumin from quarter milk samples of lactating camels using both the fluoroscan method and radial immunodiffusion test. The same author indicated that the NAGase was more reliable in predicting bacteriological status of the camel udder than serum albumin. There is a direct relationship between intra-mammary infection in cows and the conductivity of the milk produced. The test is based on the increases in sodium and chloride ions, and the consequent increase in electrical conductivity, which occurs in mastitic milk (Radostitis *et al.*, 2000). The milk conductivity was measured for both normal quarter milk samples and intra-mammary infection with *S. aureus* and *Streptococcus agalactiae* in lactating camels in Kenya (Younan *et al.*, 2001). The author explained that the conductivity reading was not diagnostic.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in three different districts namely, Negele (Borena Region), Dire Dawa, and Gewane (Afar Region) in the southern, eastern, and north eastern Ethiopia (Figure 1).

Negele (Leben District) is located in the Borena zone of the Oromia Region, southern Ethiopia (585 Km from Addis Ababa). The Borena, Arssi, and Gujji are the major ethnic groups in the area. The district has mainly a semi-arid climate. Annual mean temperatures vary from 19 to 24°C and the average annual rainfall for the area is greater than 900 mm and the rainfall is a bimodal. The altitude is below 1500 m above sea level. The area is dominated by savannah vegetation, several species of grasses and woody plants. Animal population in the region is as follows: cattle (1,77,569), goats (1,991,196), camels (669,887), donkeys (52,578), mules (6,376), and horses (507) (Peacock and Workneh, 1991; Coppock, 1994).

Dire Dawa is located 515 Km east of Addis Ababa. The area is semi-arid with a minimum of 22°C and a maximum of 34°C temperature. The rainfall pattern is a bimodal with an annual rainfall that ranges from 500 to 700 mm. The altitude varies from 500 to 1350 m above sea level. The major vegetation types in the area include short and scattered thorny trees, bushes and acacia. The major activities of the people in the region are trade and mixed farming. In Dire Dawa, the total livestock population is 183,440 goats accounting for (51.26%) of the total livestock followed by cattle (22.09%), sheep (20.66%), donkeys (3.75%), and camels (2.24%) (Dire Dawa Administrative Council Agricultural Bureau, 1998).

Gewane (Afar Region) is 269 Km from Addis Ababa. The main ethnic groups are Oromo, Amhara, and Afar. The altitude of the area varies from 150 m below sea level to 1000 m above sea level. The minimum and maximum annual temperatures are 18°C and 35°C, respectively. The region has annual rainfall (bimodal pattern) of 561 mm on the western edge of the escarpment and 225 mm on the lava plain and volcanic ash where only camels and goats are reared. Animal population in the region is comprised of cattle (700,000), sheep (100,000), goats (2,000,000), donkeys (16,000), and camels (300,000) (Wario, 1996; ANRS, 1998).

Figure 1: Study area

3.2. Study design

3.2.1. Study population and sampling method

The study animals that were sampled are traditionally managed lactating camels of different herds. Data on breed, age, stage and number of lactations, parity, previous history of mastitis, abnormalities of the udder and milk, and other data related to mastitis were recorded using a form (Appendix 1).

To determine number of clusters (herds) to be sampled when the sample size is fixed, a two-stage cluster sampling was applied. The required clusters were determined at 95% confidence level using the following formula (Thrusfield, 1995):

$$g = \frac{1.96^2 * Ts * Vc}{d^2 * Ts - 1.96^2 P_{exp}(1 - P_{exp})}$$

where

g= number of cluster to be sampled

P_{exp}= expected prevalence

d= desired absolute precision

Ts= total number of animals to be sampled

Vc= between-cluster variance

Determination of expected prevalence (P_{exp}) depended on previous information on camel mastitis in Ethiopia, Almaw and Molla (2000), Salah (2000), and Bekele and Molla (2001). These investigators reported prevalence of clinical and sub-clinical mastitis in the camels as 24.1%, 48.1%, and 12.1% respectively. Based on these an average of 28.1% was obtained. The desired absolute precision (d) of 10% was used. A total number of quarter milk samples (Ts) of 1012 (500, 320, and 192 quarter milk samples for Negele (Borena Region), Dire Dawa, and Gewane (Afar Region) were obtained, respectively. There was no information available on cluster samples from previous reports, thus the estimation of between-cluster variance (V_c) was done by estimating standard deviation (the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence). The between-cluster variance (V_c) was

0.085. Thus, 35 clusters were sampled (28 clusters for Negele (Borena Region), 4 clusters for Dire Dawa, and 3 clusters for Gewane (Afar Region). The term cluster refers to the herd. The herd size in the study sites varied from one camel up to twelve camels.

3.2.2. Questionnaire survey

Data on knowledge related to mastitis, the types and use of anti-suckling devices, practices of cauterization as means of treating udder problems, tick infestations problem and methods of their control, and other camel management practices were obtained by means of a questionnaire from the owners of the camel during each visit (Appendix 2). The questionnaire survey was done in the three study sites and was based on the willingness of camel herd owners.

3.2.3. Collection of quarter milk samples

The suckling calf was used to stimulate milk let-down. Before the collection of quarter milk samples, the teats were disinfected with cotton wool moistened with 70% ethyl alcohol. The first few squirts of milk were discarded and about 5-20 ml collected in a sterile universal bottle. The quarter milk samples were kept in an ice-box and transported as soon as possible to the laboratory at the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit. In the laboratory the quarter milk samples were kept at -20°C before the application of the California Mastitis Test (CMT), Somatic Cell Counts (SCC), and microbiological investigations.

3.2.4. California Mastitis Test (CMT)

The California Mastitis Test (CMT) was carried out using the method described by Schalm *et al.* (1971) and Quinn *et al.* (1994). Briefly, equal volumes of commercial CMT reagent and quarter milk were mixed. The interpretation of the result was done as described by Quinn *et al.* (1994), Table 1. For the analysis, negative (0) and trace (±) were considered as negative and different intensities of positive (1, 2, and 3) were considered as positive.

Table 1: Interpretation of CMT results and relationship with SCC

CMT score	Interpretation	Visible reaction	Total cell count
0	Negative	Milk fluid and normal	0-200,000 0-25% Neutrophil
±	Trace	Slight precipitation	150,000-500,000 30%-40% Neutrophil
1	Weak positive	Distinct precipitation but no gel formation	400,000-1,500,000 40%-60% Neutrophil
2	Distinct positive	Mixture thickness with a gel formation	300,000-5,000,000 60%-70% Neutrophil
3	Strong positive	Viscosity greatly increased strong gel that is cohesive with a convex surface	≥ 5,000,000 70%-80% Neutrophil

Source: Quinn *et al.* (1994)

3.2.5. Somatic Cell Count (SCC)

The Direct Microscopic Somatic Cell Counts (DMSCC) was applied as recommended by Packard *et al.* (1992). The following procedure was employed:

- The magnification factor (MF) was determined in order to calculate the total number of SCC per ml using the following formula:

$$\text{Magnification factor (MF)} = \frac{40000}{3.1416 \times d^2}$$

where d =diameter

- Milk sample (0.01 ml) was spread over an area of 1 cm^2 of a glass slide. The milk samples were warmed immediately before transferring them to slides.
- The smears were dried and heated slowly to prevent from cracking and peeling.
- The smears were stained with Levowitz Weber modification of the Newman Lampert stain for two minutes (the stain was placed in a container covered to prevent evaporation).
- The total number of cell counts per ml was calculated using the following formula:

$$\text{Number of leukocyte per ml of milk} = \frac{\text{Number of leukocytes counted} \times \text{MF}}{\text{Number of fields counted}}$$

The somatic cell counts (SCC) of 2.5×10^5 cell ml^{-1} was used as the cut-off point (Radostitis *et al.*, 2000) to classify quarter milk samples of the lactating camel as positive in the analysis.

3.2.6. Bacteriological procedures

Frozen quarter milk samples were thawed at room temperature. The bacteriological culture was performed following the standard microbiological techniques (Quinn *et al.*, 1994). One loopful of milk was streaked on 5% sheep blood agar and MacConkey agar to detect Gram-negative bacteria could grow on this medium. The plates were incubated aerobically at 37°C for 24 and 48 hours. The plates were examined for growth, morphologic features of the colonies, and hemolytic characteristics. The following assumption was applied for the interpretation of the results:

- A sample was considered positive if one of the known mastitis causative agents was isolated.
- A sample was considered negative if there was no growth after 48 hours.
- A sample was considered contaminated if there was profuse growth of environmental bacteria.

Presumptive identification of bacteria on primary culture was done on the basis of colony morphology, hemolytic characteristics, Gram-stain and biochemical tests. *Staphylococcus spp.* and *Micrococcus spp.* were identified based on catalase test, coagulase test, haemolysis, pigment production, fermentation of maltose (purple agar + 1% maltose). Presence of *Streptococcus spp.* and *Enterococcus faecalis* was determined according to CAMP reaction, type of haemolysis, growth characteristic on Edward's medium, and sugar fermentation. *Corynebacterium spp.* and

Bacillus cereus were identified based on hemolytic characteristics, catalase test, growth on 9% NaCl, CAMP reaction, and sugar fermentation tests. Gram-negative isolates were idendified based on growth on MacConkey agar, catalase test, oxidase reaction, triple sugar iron agar (TSI), IMViC test, urease and sugar fermentation tests. The differentiation of microbial isolates was carried out as summarized in Appendix 3.

Case definition: An individual mammary quarter was defined as non-infected (NI) if no microorganism was isolated, infected with major pathogens (MAP) if *Staphylococcus aureus, Streptococcus agalactiae, S. uberis, Enterococcus faecalis, Micrococcus spp, Actinomyces pyogenes, Bacillus cereus, Pasteurella haemolytica,* and coliforms were present, and infected with minor pathogens (MIP) if coagulase-negative Staphylococci *(CNS), Corynebacterium bovis,* and other environmental pathogens were isolated. This classification was based on the presence of pathogens which are responsible for udder infection (Quinn *et al.*, 1994; Abdurahman *et al.*, 1995; Obied *et al.*, 1996; El-Jakee, 1998; Radostits *et al.*, 2000; Bekele and Molla, 2001).

3.2.7. Determination of clinical and sub-clinical mastitis

Clinical mastitis was recognized by abnormal milk and signs of udder infection (abnormalities of the udder). Sub-clinical mastitis refers to the existence of inflammation of the udder in the absence of gross signs. This was established by bacteriological examinations (major pathogens). The following formula (Thrusfield, 1995) was used for the calculations of both the prevalence of clinical and sub-clinical mastitis in camels at the quarter level:

$$\text{Prevalence} = \frac{\text{No. of quarter milk samples with mastitis}}{\text{Total number of quarter milk samples}}$$

3.2.8. Anti-microbial susceptibility test

Susceptibility levels of bacteria isolated from camel mastitis to commonly used anti-microbials were estimated using the single disc diffusion susceptibility test (Kirby-Bauer Method) (NCCLS, 1990, 1997; Quinn *et al.*, 1994). Briefly, this was performed as follows:

- The isolates were transferred to a tube containing 5 ml tryptic soy broth. The mixture was incubated at 37°C until a slight visible turbidity appeared. This was compared with the McFarland 0.5 turbidity standard.

- The isolates were streaked onto Mueller-Hinton agar which contained 5% defibrinated blood.

- McFarland 0.5 turbidity standard was prepared by mixing 0.5 ml of solution A (0.048 M $BaCl_2$) and 99.5 ml of solution B (0.36 N H_2SO_4).

Solution A (0.048 M $BaCl_2$): 1.75 gram $BaCl_2X2H_2O$ was diluted in 100 ml distilled water.

Solution B (0.36 N H_2SO_4): 1 ml H_2SO_4 was mixed with 100 ml distilled water.

- The antibiotic discs were incubated for 18 to 24 hours at 37°C and the diameters of zone of growth-inhibition were measured in millimeters and reported as:

1- Susceptible: Refers to the response of the pathogens to the anti-microbial agent at the normal dosage.

2- Moderately susceptible: The possibility of the pathogens inhibited by attainable concentration of the anti-microbial agent provided a higher dosage was used.

3- Intermediate: The pathogens were not fully susceptible to the specific anti-microbial agent.

4- Resistant: The pathogens were not inhibited by the usually achievable systemic concentration of the anti-microbial agent.

Interpretations of the inhibition zone sizes of anti-microbial agents are summarized in Table 2.

Table 2: Zone size interpretation of anti-microbial agents

Anti-microbial agent	Disc code	Disc content	Interpretation of zone size in mm			
			Resistant	Intermediate	Moderately susceptible	Susceptible
Ampicillin						
Staphylococci			≤ 28	-	-	≥ 29
non-enteric *Streptococci*	AP	10 µg	≤ 21	-	22-29	≥ 30
Enterococci			≤ 16	-	≥ 17	-
Enterobacteriaceae			≤ 13	-	14-16	≥ 17
Other organisms			≤ 13	-	14-17	≥ 18
Erythromycin	E	15 µg	≤ 13	14-22	-	≥ 23
Kanamycin	K	30 µg	≤ 13	14-17	-	≥ 18
Penicillin G						
Staphylococci			≤ 28	-	-	≥ 29
Streptococci	PG	10 Units	≤ 19	-	20-27	≥ 28
Enterococci			≤ 14	-	-	≥ 15
Other organisms			≤ 16	-	-	≥ 17
Streptomycin	S	10 µg	≤ 11	12-14	-	≥ 15
Tetracycline	T	30 µg	≤ 14	15-18	-	≥ 19
Oxytetracycline	OT	30 µg	≤ 14	15-18	-	≥ 19
Chloramphenicol	C	30 µg	≤ 12	13-17	-	≥ 18
Gentamicin	GM	10 µg	≤ 12	13-14	-	≥ 15
Nalidixic acid	NA	30 µg	≤ 13	14-18	-	≥ 119

Source: Modified from NCCLS (1990 and 1997) and Quinn *et al.* (1994)

3.2.9. Data management and analysis

Microsoft Excel (Windows 1997), Statgraphics plus for Windows 2.1, and Stata 6.0 for Windows 98/95/NT were used for data analysis. Descriptive statistics were used for all the variables. Chi-square (χ^2) was used for assessing the statistical associations of various risk factors with mastitis. The logistic regression model was employed to obtain the odds ratios between different factors and occurrence of camel mastitis. Interpretation of the results was done as follows: exposure could not be associated with mastitis if the odds ratio (OR) =1, could be a risk factor if the OR > 1, and could be a protective factor if the OR < 1. To determine the significance of the association, 95% CI was used. For instance, the association between risk factor and occurrence of camel mastitis was considered statistically significant if the 95% CI did not include one.

Kappa statistic was used to assess the agreements between CMT, SCC, and bacteriological results, since there was no gold standard test for the calculation of sensitivity and specificity (Thrusfield, 1995). The cut-off levels which were used in the test were:

Microbiological examinations based on: MAP = +ve MIP = -ve

CMT based on: 1, 2, 3 = +ve Trace (±) & negative (0) = -ve

SCC based on: $\geq 2.5 \times 10^5$ ml^{-1} = +ve $< 2.5 \times 10^5$ ml^{-1} = -ve

The logic of using this approach was that agreement between tests was regarded as evidence of validity. Kappa statistic ranges from 1 (complete agreement) to 0 (no agreement). Negative values indicated that agreement was less than expected by chance. The other point estimates according to Thrusfield (1995) are:

0-0.20: slight agreement

0.21-0.40: fair agreement

0.41-0.60: moderate agreement

0.61-0.80: substantial agreement

> 0.81: almost perfect agreement

Kruskall Wallis was used to compare medians log SCC to CMT scores and bacteriological examinations (MAP, MIP, and NI). The log SCC was not normaly distributed after transformation. Therefore, Kruskall Wallis, a non-parametric test, was employed instead of using the parametric ANOVA.

4. RESULTS

4.1. Questionnaire survey

Questionnaire survey was done in order to have some background about the knowledge of camel mastitis, milking and related problems as well as management practices such as use of anti-suckling devices, cauterization, and other means of control of camel mastitis. To achieve these objectives 20 owners of camel herds (11, 6, and 3 owners from Negele (Borena Region), Dire Dawa, and Gewane (Afar Region), respectively) were interviewed. The responses to the questionnaire survey are summarized in Table 3. The results showed that the main purposes for keeping camels were for milk and meat production and source of income (50%, n = 10). Nine owners (45%) selected only milk and meat production. Nineteen owners (95%) explained that diseases and feed shortage together with water scarcity were the major constraints of camel rearing. The same number of owners confirmed the presence of udder problems. However, 13 owners (65%) did not know any type of mastitis treatment. Thirteen (65%) and 12 (60%) owners confirmed practice of cauterization and use of anti-suckling devices, respectively. High frequency of milking, three times (50%, n = 10) and four times (40%, n = 8) were recorded in the interview. Twenty (100%) owners did not clean the udder, but 11 (55%) washed their hands before milking.

4.2. Cross-sectional study

One thousand and twelve udder quarters from 253 traditionally managed lactating camels (*Camelus dromedarius*) were investigated according to multi-stage sampling in the study sites. Out of the 1012 udder quarters, 35 (3.46%) quarters were blind and 21 (2.08%) quarter milk samples were lost during handling. The remaining 956 quarter milk samples (466, 305, 185 from Negele (Borena Region), Dire Dawa, and Gewane (Afar Region), respectively) were tested for California Mastitis Test (CMT), Somatic Cell Counts (SCC), and bacteriological analysis. The results are given in Table 5.

Table 3: Summary of the questionnaire survey responses by camel herd owners in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region)

	A	B	C	Total
		n (%)		
Purpose for keeping camels				
a. milk & meat production	5 (45.45)	3 (50.00)	1 (33.33)	9 (45.00)
b. income	0.00	0.00	0.00	0.00
c. both	5 (45.45)	3 (50.00)	2 (66.67)	10 (50.00)
d. other	1 (9.09)	0.00	0.00	1 (5.00)
The constraints of camel rearing				
a. feed shortage & water scarcity	0.00	1 (16.67)	0.00	1 (5.00)
b. diseases	0.00	0.00	0.00	0.00
c. both	11 (100.00)	5 (83.33)	3 (100.00)	19 (95.00)
Presence of mastitis				
a. Yes	11 (100.00)	5 (83.33)	3 (100.00)	19 (95.00)
b. No	0.00	1 (516.67)	0.00	1 (5.00)
Cause of mastitis				
a. tick	6 (54.54)	2 (33.33)	3 (100.00)	11 (55.00)
b. flies	0.00	0.00	0.00	0.00
c. both	5 (45.45)	0.00	0.00	5 (25)
d. nothing	0.00	4 (66.67)	0.00	4 (20)
Types of treatment				
a. antibiotic	1 (9.09)	0.00	0.00	1 (5.00)
b. traditional	4 (36.36)	2 (33.33)	0.00	6 (30.00)
c. nothing	6 (54.54)	4 (66.67)	3 (100.00)	13 (65.00)
Practice of cauterization				
a. Yes	7 (63.64)	3 (50.00)	3 (100.00)	13 (65.00)
b. No	4 (36.36)	3 (50.00)	0.00	7 (35.00)
Use of anti-suckling devices				
a. Yes	11 (100.00)	0.00	1 (33.33)	12 (60.00)
b. No	0.00	6 (100.00)	2 (66.67)	8 (40.00)
Tick control				
a. acaricides	2 (18.18)	2 (33.33)	0.00	4 (20.00)
b. manual removal	1 (9.09)	0.00	0.00	1 (5.00)
c. both	7 (63.64)	4 (66.67)	3 (100.00)	14 (70.00)
d. other	1 (9.09)	0.00	0.00	1 (5.00)
Frequency of milking				
a. once	1 (9.09)	0.00	0.00	1 (5.00)
b. twice	0.00	0.00	1 (33.33)	1 (5.00)
c. three times	7 (63.64)	2 (33.33)	1 (33.33)	10 (50.00)
d. four times	3 (27.27)	4 (66.67)	1 (33.33)	8 (40.00)
Cleaning the udder before milking				
a. Yes	0.00	0.00	0.00	0.00
b. No	11 (100.00)	6 (100.00)	3 (100.00)	20 (100.00)
Washing the hands before milking				
a. Yes	3 (27.27)	6 (100.00)	2 (66.67)	11 (55.00)
b. No	8 (72.73)	0.00	1 (33.33)	9 (45.00)

A = Negele (Borena Region) B = Dire Dawa C = Gewane (Afar Region)

n = number of owners (%) = percentage of owners

Table 1: The distribution and percentage of the results of California Mastitis Test (CMT), Somatic Cell Counts (SCC), and bacteriological examinations

Unit	Quarter milk samples				Total
	RF	RR	LF	LR	
	n (%)				
Site					
Negele (Borena Region)	114 (47.70)	117 (49.16)	119 (48.97)	116 (49.15)	466 (48.74)
Dire Dawa	78 (32.64)	75 (31.51)	77 (31.69)	75 (31.78)	305 (31.90)
Gewane (Afar Region)	47 (19.67)	46 (19.33)	47 (19.34)	45 (19.07)	185 (19.35)
Sub-total	239 (100.00)	238 (100.00)	243 (100.00)	236 (100.00)	956 (100.00)
Site (all)					
Blind	11 (4.60)	10 (4.20)	7 (2.88)	7 (2.95)	35 (3.46)
Missing	3 (1.26)	5 (2.10)	3 (1.23)	10 (4.22)	21 (2.08)
Bacteriological examinations (all sites)					
Major pathogens (MAP)	96 (40.68)	99 (42.31)	102 (42.68)	131 (53.04)	428 (44.77)
Minor pathogens (MIP)	46 (19.49)	36 (15.38)	41 (17.15)	20 (8.10)	143 (14.96)
Non-infected (NI)	94 (39.83)	99 (42.31)	96 (40.17)	96 (38.87)	385 (40.27)
Sub-total	236 (100.00)	234 (100.00)	239 (100.00)	247 (100.00)	956 (100.00)
California Mastitis Test (CMT) (all sites)					
Negative	109 (46.19)	104 (45.22)	125 (52.52)	102 (44.54)	800 (85.74)
Trace (±)	88 (37.29)	101 (43.91)	78 (32.77)	93 (40.61)	
Positive	39 (16.53)	25 (10.87)	35 (14.71)	34 (14.85)	133 (14.26)
Sub-total	236 (100.00)	230 (100.00)	238 (100.00)	229 (100.00)	933* (100.00)
Somatic Cell Counts (SCC) (all sites)					
$\geq 2.5 \times 10^5$ ml^{-1}	71 (33.02)	61 (30.35)	78 (37.50)	61 (29.76)	271 (32.69)
$< 2.5 \times 10^5$ ml^{-1}	144 (66.98)	140 (69.65)	130 (62.50)	144 (70.24)	558 (67.31)
Sub-total	215 (100.00)	201 (100.00)	208 (100.00)	205 (100.00)	829* (100.00)

RF: right front RR: right rear LF: left front LR: left rear
n: number of samples (%): percentage of samples
*: some samples were lost during handling in the laboratory

4.2.1. Prevalence of clinical and sub-clinical mastitis

Prevalences of both clinical and sub-clinical mastitis are given in Table 5. High prevalence of sub-clinical mastitis was recorded from quarter milk samples in Dire Dawa and Gewane (Afar Region) as 46.2% (n = 141) and 45.4% (n = 84), respectively. However, there were no clinical cases recorded in these two areas. Sub-clinical mastitis was present in 173 (37.1%) quarter milk samples examined from Negele (Borena Region). Only 5 (1%) quarters had clinical mastitis in this area.

Table 5: The prevalence of clinical and sub-clinical mastitis obtained in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region)

Source of quarter milk samples	No. of quarter milk samples	Clinical mastitis [a]	Sub-clinical mastitis [b]
		No. of positive (prevalence %)	
Negele (Borena Region)	466	5 (1.1%)	173 (37.1%)
Dire Dawa	305	0.0	141 (46.2%)
Gewane (Afar Region)	185	0.0	84 (45.4%)
Over all	**956**	**5 (0.5%)**	**398 (41.6%)**

a: clinical mastitis based on the inspection of the udder and milk

b: sub-clinical mastitis based on the results of bacteriological examination

Major pathogens (MAP) = +ve

Minor pathogens (MIP) and non-infected (NI) = -ve

4.2.2. Intra-mammary infection

A total of 956 quarter milk samples from 253 traditionally managed lactating camels were collected aseptically and examined using standard microbiological techniques irrespective of the results of California Mastitis Test (CMT) and somatic cell counts (SCC). Five hundred and seventy one (59.73%) quarter milk samples had microorganisms and 385 (40.27) quarter milk samples were found negative for microbial analysis. Out of 571, 428 (75.00%) isolates were identified as major pathogens (MAP) and 143 (25.00%) isolates were considered minor pathogens (MIP) (Table 6).

Proportionally, the major pathogens (MAP) isolated from sub-clinical mastitis were as follows: *S. aureus* 24.7% (n = 139),*E. coli* (17.4%, n = 98), *Enterobacter aerogenes* (9.6%, n = 54), *Bacillus cereus* (9.1%, n = 51), and *Streptococcus uberis* (6.8%, n = 38) of the total isolates of sub-clinical mastitis. Coagulase-negative Staphylococci (CNS) (*S. hyicus* and *S. epidermidis*) were the common minor (MIP) pathogens. They accounted for 9.6% (n = 54) and 9.1% (n =51) of the total microorganisms of sub-clinical mastitis, respectively. The microorganisms isolated from clinical cases were *S. aureus* 22.2% (n = 2), *S. hyicus* 22.2% (n = 2), *Streptococcus uberis* 22.2% (n = 2), *Bacillus cereus* 11.1% (n = 1), *E: coli* 11.1% (n = 1), and *S. epidermidis* 11.1% (n = 1). These were isolated from 5 quarter milk samples collected in Negele (Borena Region).

4.2.3. California Mastitis Test (CMT)

For the analysis, CMT scores of negative (0) and trace (±) were considered as negative and different intensities of CMT positive (1, 2, 3) were considered as positive. A significant correlation (χ^2 = 49.98, *P*-value = 0.00) was found between CMT scores and bacteriological groupings (MAP, MIP, and NI). Out of 933 quarter milk samples examined, 133 (14.26%) were CMT positive (some quarter milk samples were tested only for SCC and microbial analysis, as the amount of the milk was not enough for CMT). The CMT positive cases were associated with quarter milk samples that had major pathogens and minor pathogens (81 (60.90 %) and 37 (27.82%), respectively). However, 15 (11.28%) CMT positive cases were from non-infected (NI) quarter milk samples. Analysis of the different levels of CMT scores in relation to the bacteriological examinations indicated that the differences were statistically significant. The percentages of CMT scores classified by bacteriological groupings are presented in Figure 2.

Table 6: Frequency and percentage of the bacteria isolated from camel mastitis in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region)

Isolates*	Clinical mastitis	Sub-clinical mastitis	Total
	n (%)		
Major pathogens (MAP)			
Staphylococcus aureus	2 (22.2)	139 (24.7)	141 (24.6)
Streptococcus agalactiae	0.0	15 (2.7)	15 (2.6)
Str. uberis	2 (22.2)	38 (6.8)	40 (7.0)
Enterococcus faecalis	0.0	3 (0.5)	3 (0.5)
Micrococcus spp.	0.0	3 (0.5)	3 (0.5)
Actinomyces pyogenes	0.0	4 (0.7)	4 (0.7)
Bacillus cereus	1 (11.1)	51 (9.1)	52 (9.1)
Pasteurella haemolytica	0.0	12 (2.1)	12 (2.1)
E. coli	1 (11.1)	98 (17.4)	99 (17.3)
Klebsiella pneumoniae	0.0	5 (0.9)	5 (0.9)
Enterobacter aerogenes	0.0	54 (9.6)	54 (9.5)
Sub-total	**6 (66.7)**	**422 (75.1)**	**428 (75.0)**
Minor pathogens (MIP)			
S. hyicus	2 (22.2)	54 (9.6)	56 (9.8)
S. epidermidis	1 (11.1)	51(9.1)	52 (9.1)
S. intermedius	0.0	5 (0.9)	5 (0.9)
Str. pyogenes	0.0	6 (1.1)	6 (1.1)
Str. pneumonae	0.0	2 (0.4)	2 (0.4)
Corynebacterium bovis	0.0	8 (1.4)	8 (1.4)
Aeromonas hydrophila	0.0	6 (1.1)	6 (1.1)
Serratia marcescens	0.0	3 (0.5)	3 (0.5)
Proteus mirabilis	0.0	3 (0.5)	3 (0.5)
Proteus vulgaris	0.0	2 (0.4)	2 (0.4)
Sub-total	**3 (33.3)**	**140 (24.9)**	**143 (25.0)**
Total	**9 (100.0)**	**562 (100.0)**	**571 (100.0)**

*: The isolates from all regions n: number of isolates (%): percentage of isolates

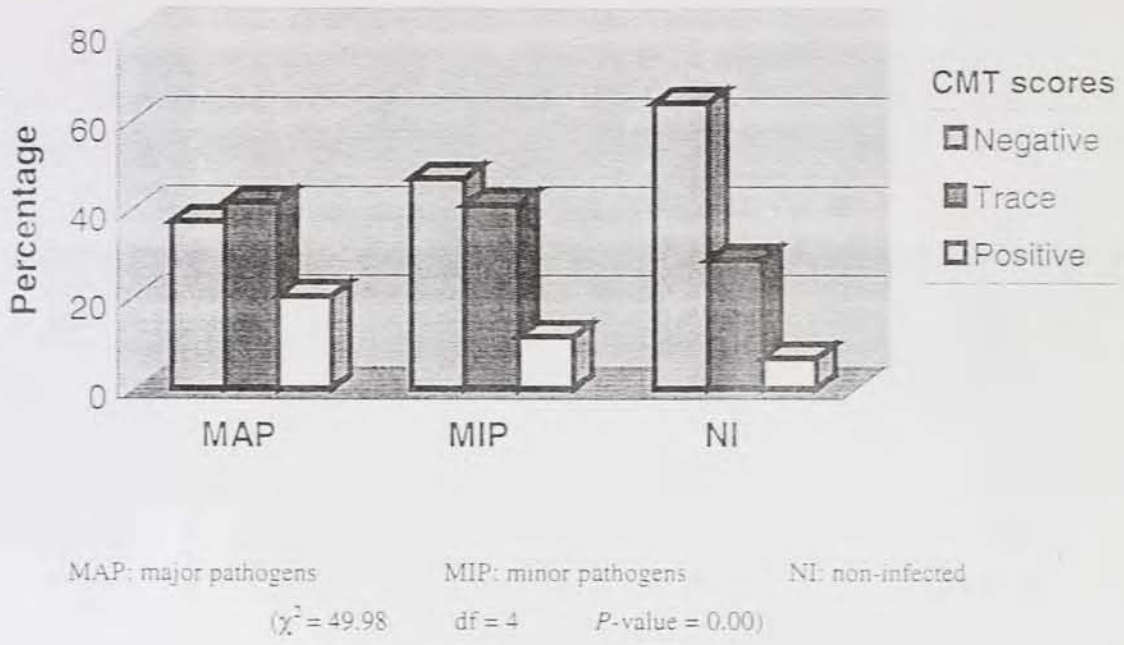


Figure 2: The percentage of CMT scores in relation to bacteriological groupings

With regard to somatic cell counts (SCC), for the analysis SCC of 2.5 x 10^5 ml^{-1} was used as cut-off value to classify quarter milk samples as positive and negative. There was a significant association (χ^2 = 28.23 *P*-value = 0.00) between CMT scores and SCC (Table 7).

For the evaluation of CMT, the lactation periods were classified into three groups: first stage (1-120 days), second stage (121-240 days), and third stage (> 240 days). A significant agreement (χ^2 = 25.14, *P*-value = 0.00) was found between CMT scores and stages of lactation (Figure 3). Out of 933 quarter milk samples examined, most of the CMT positive samples were from the first stage of lactation 44.36% (n = 59) followed by the second stage 37.59% (n = 50) and the third stage 18.05% (n = 24). Similar findings were recorded for the lactation categories of the CMT trace (±) results as 45.66% (n = 163), 29.41% (n = 105), and 24.92% (n = 89) for the first, second, and third stages of lactation, respectively). Further analysis for each stage of lactation in relation to CMT scores, a significant differences of CMT scores were observed only for the second and third stages of lactation (χ^2 = 13.40 and 20.73; *P*-value = 0.001 and 0.000, respectively).

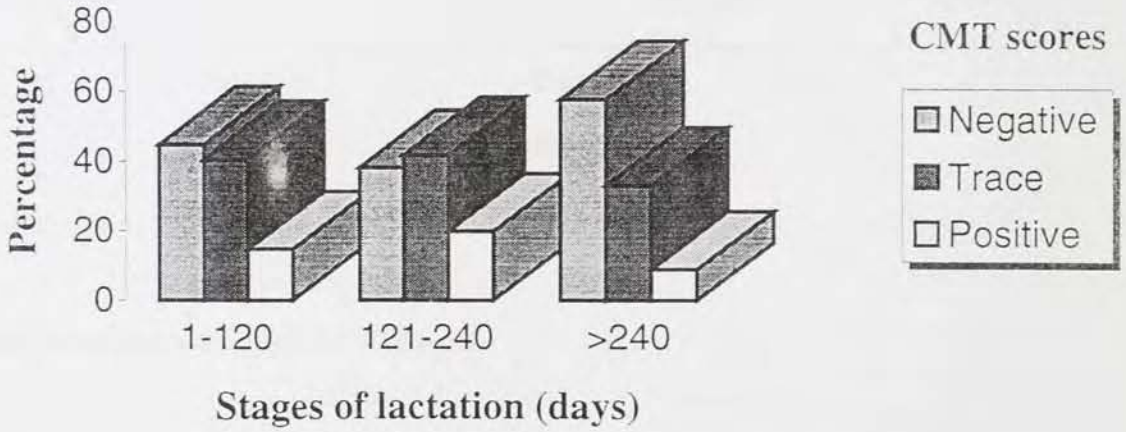
Table 7: Distribution and (%) of CMT scores according to somatic cell counts cut-off level 2.5×10^5 ml^{-1}

Cut-off level	CMT scores			Total
	Negative	Trace (±) n (%)	Positive	
≥ 2.5×10^5 ml^{-1}	89 (33.3)	112 (41.9)	66 (24.7)	267 (100.0)
< 2.5×10^5 ml^{-1}	260 (43.3)	277 (46.2)	63 (10.5)	600 (100.0)

n: number of quarter milk samples (%): percentage of quarter milk samples

Cut-off level: ≥ 2.5×10^5 ml^{-1} = +ve < 2.5×10^5 ml^{-1} = -ve

($\chi^2 = 28.23$ df = 2 *P*-value = 0.00)



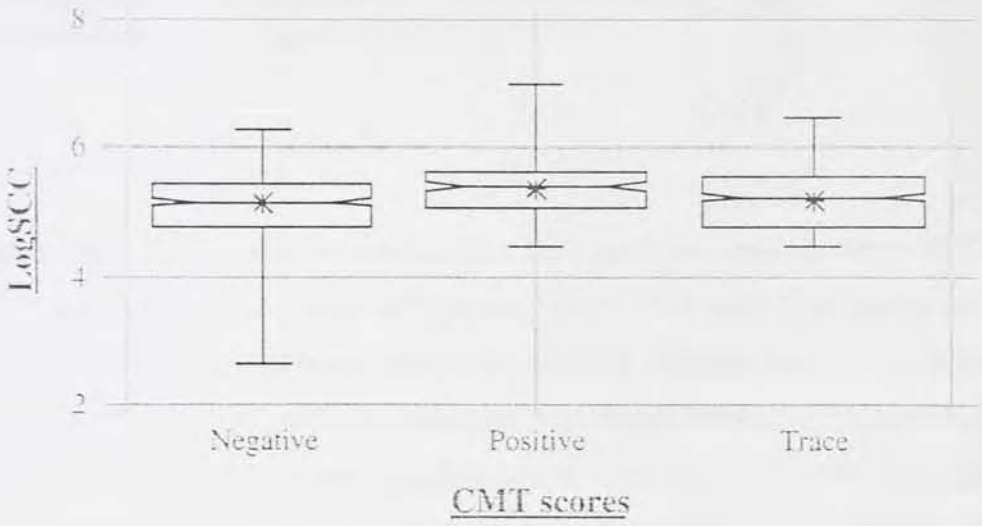
($\chi^2 = 25.14$ df = 4 *P*-value = 0.00)

Figure 3: The percentage of CMT scores throughout the lactation periods

4.2.4. Somatic Cell Counts (SCC)

The somatic cell counts (SCC) were expressed in logarithmic scale (log_{10}) for the purpose of statistical analysis. The medians log SCC of CMT positive samples were significantly different (*P*-value = 0.00) from the medians log SCC of CMT negative and trace (±) samples. The results are presented in Figure 4.

Similar procedure was applied for bacteriological results. The differences among the medians log SCC of bacteriological groupings of major pathogens (MAP), minor pathogens (MIP), and non-infected (NI)) were not significant (Kruskall Wallis = 2.868, *P*-value = 0.238) (Table 8). The application of the cut-off level of 2.5 x 10^5 ml^{-1} indicated less agreement (χ^2 = 2.29, *P*-value = 0.319).



The logSCC is not normaly distributed after transformation.

(Kruskall Wallis = 18.50 *P*-value = 0.00)

Figure 4: Log SCC for each CMT score

Table 8: Descriptive statistic of log SCC* for bacteriological groupings

	n	Median	Mean	SD	SE	CI (95%)
MAP	351	5.24	5.24	0.48	0.03	5.21 – 5.28
MIP	266	5.24	5.19	0.45	0.03	5.15 – 5.23
NI	174	5.20	5.18	0.48	0.04	5.13 – 5.22

MAP: major pathogens MIP: minor pathogens NI: non-infected

*: The logSCC was not normaly distributed after transformation n: number of quarter milk samples examined

SD: standard deviation SE: standard error CI: confidence interval

Kruskall Wallis = 2.868 *P*-value = 0.238

Chi-square (χ^2) test was selected to determine the significant levels between SCC cut-off of 2.5 x 10^5 ml^{-1} and varios factors (stage of lactation, parity, and age). The results are summarized in Table 9. There was no significant association between different stages of lactation and SCC cut-off of 2.5 x 10^5 ml^{-1}. A significant agreement was found between SCC cut-off of 2.5 x 10^5 ml^{-1} and parity of 3 - 4 and 5 - 6 and age group of 4 - 6 years. To quantify this agreement, logistic regression model was used. The results showed that the parity of 5 - 6 could be a risk factor (OR= 1.447) for SCC cut-off of 2.5 x 10^5 ml^{-1} (95% CI = 0.487 - 0.911) (Table 10). While, the parity of 3 - 4 and age group of 4 - 6 years were significantly protective factors for SCC cut-off of 2.5 x 10^5 ml^{-1} (OR= 0.666 and 0.501; 95% CI = 1.010 - 2.073 and 0.323 - 0.777, respectively).

Table 9: The associations between somatic cell counts cut-off level 2.5 x 10^5 ml^{-1} and various factors

Factors	χ^2	*P*- value
Stage of lactation (days):		
1 - 120	0.01	0.909
121 - 240	1.19	0.276
> 240	0.97	0.325
Number of parity:		
1 - 2	0.25	0.616
3 - 4	6.52	0.011*
5 - 6	4.08	0.043*
> 6	0.40	0.529
Age (years):		
1 – 3	-	-
4 – 6	9.78	0.002**
7 – 9	3.52	0.061
9	0.42	0.519

The results for all regions

χ^2: Chi-square *P*-value: significant level

*: *P*-value < 0.05 **: *P*-value < 0.01

Table 10: Logistic regression model selected to demonstrate associations between S CC cut-off level 2.5 x 10^5 $ml^{-1}$and parity of 3 - 4 and 5 - 6 and age group of 4 - 6 years

Factor	Coef.	SE	Z	OR (95% CI)
Number of parity:				
3 - 4	-0.407	0.160	-0.546	0.666 (0.486-0.911)
Constant	0.992	0.132	7.529	-
5 – 6	0.427	0.183	2.014	1.447 (1.010-2.073)
Constant	0.427	0.163	2.614	-
Age (years):				
4 - 6	-0.691	0.224	-3.084	0.501 (0.323-0.777)
Constant	1.315	0.209	6.287	-

Coef. = coefficient SE = standard error of the coefficent Z = Coef./SE
OR = odd ratio CI = confidence interval

4.2.5 Agreement between tests (Kappa statistic)

Kappa statistic was used to assess the agreements between the different tests (CMT, SCC, and bacteriological examinations). The points estimates of the test for bacteriological examinations and CMT; bacteriological examinations and SCC; and CMT and SCC were 0.12, 0.04, and 0.15, respectively (Table 11). Significant associations (*P*-value = 0.00) between CMT and both bacteriological examinations and SCC were obtained, but no significant agreement (*P*-value = 0.238) between bacteriological examinations and SCC was recorded.

Table 11: Agreements between different tests (Kappa statistic)

Test	Agreem. (%)	Exp. Agreem. (%)	Kappa
Bacteriological examinations & CMT	61.15	55.77	0.12[a]
Bacteriological examinations & SCC	53.92	51.98	0.04[a]
CMT & SCC	67.77	61.92	0.15[a]

CMT: California Mastitis Test
SCC: Somatic Cell Counts
Agreem. = agreement
Exp. = expected
a: indicates a slight agreement
(%): the percentage

Cut-off levels

Bacteriological examinations based on: major pathogens (MAP) = +ve minor pathogens (MIP) = -ve
CMT based on: 1, 2, 3 = +ve Trace (±) & negative (0) = -ve
SCC based on: $\geq 2.5 \times 10^5$ ml^{-1} = +ve $< 2.5 \times 10^5$ ml^{-1} = -ve

4.2.6. Risk factors analysis

There was no statistical significance between the occurrence of camel mastitis and various risk factors (tick infestation, teat lesions, conformation of the udder, previous history of mastitis, and stage of lactation, parity, and age) (Table 12). A positive correlation (χ^2 =7.77; *P*-value = 0.005) was only recorded between the use of anti-suckling devices and occurrence of camel mastitis. Logistic regression model showed that anti-suckling devices could be a risk factor for camel mastitis (OR= 1.807; 95% CI=1.207-2.698).

Table 12: The association between occurrence of camel mastitis and some risk factors

Factors	χ^2	*P*- value
Tick infestation	0.00	1.000
Teat lesion	0.26	0.612
Conformation of the udder	2.60	0.107
Previous history of mastitis	0.05	0.826
Anti-suckling devices[a]	7.77	0.005**
Stage of lactation (days) :		
1 - 120	0.09	0.760
121 - 240	0.02	0.880
240	0.03	0.853
Number of parity:		
1 - 2	1.16	0.282
3 - 4	0.19	0.667
5 - 6	0.75	0.386
6	0.01	0.905
Age (years):		
1 – 3	-	-
4 – 6	0.31	0.578
7 – 9	0.41	0.522
> 9	0.99	0.320

The results for all regions except the anti-suckling devices only for Negele, (Borena Region)

χ^2: Chi-square *P*-value: significant level **: *P*-value < 0.01

a: anti-suckling devices: odds ratio (OR) =1.807 95% confidence interval (CI) = (1.207-2.698)

4.2.7. In-vitro anti-microbial susceptibility test

Out of 571 pathogens isolated from intra-mammary infection of lactating camel, 219 isolates of major and minor pathogens were subjected to in-vitro anti-microbial susceptibility test using the single disc diffusion method. The drugs used were kanamycin, oxytetracycline, tetracycline, penicillin G, gentamicin, ampicillin, cloramphenicol, streptomycin, erythromycin, and nalidixic acid.

Staphylococcus aureus isolates were found to be 100% susceptible to kanamycin, tetracycline, cloramphenicol, gentamicin, and streptomycin (Table 13). They were also highly susceptible to oxytetracyclin (97.1%), ampicillin (94.3%), and penicillin G (91.4%). *S. hyicus*, *S. intermedius*, and *Micrococcus spp.* isolates were very sensitive (100%) to oxytetracycline, kanamycin, tetracycline, cloramphenicol, gentamicin, and streptomycin. Similar percentage was recorded for *S. epidermidis* against cloramphenicol and gentamicin. *S. aureus*, *S. epidermidis*, *S. intermedius*, and *Micrococcus spp.* isolates were high resistance (100%) against nalidixic acid.

Oxytetracycline and tetracycline were drugs of choice against 10 isolates of *Streptococcus agalactiae* followed by cloramphenicol (90%) and penicillin G (80%). *S. uberis* isolates were found sensitive to gentamicin, cloramphenicol, oxytetracycline, and tetracycline (92.9%, 85.7%, 78.6%, and 71.4%, respectively).

Gentamicin was an effective antibiotic (100%) against *Corynebacterium bovis* and *Actinomyces pyogenes*. High sensitivity of the two isolates was also recorded for chloramphenicol (87.5% and 100% respectively). *Pasteurella haemolytica* and *Bacillus cereus* isolates were found sensitive to gentamicin (100%, 90%, respectively) and kanamycin (87.5% and 80%, respectively). Enterobacteriaceae were found susceptible to gentamicin, chloramophenicol, kanamycin, and oxytetracycline. However, *Enterobacter aerogenes* and *Serratia marcescens* showed low susceptibility to oxytetracycline (30% and 66%, respectively). Erythromycin and penicillin G were found 100% resistant against *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Serratia marcescens*.

Table 13: Antimicrobial susceptibility test* of bacteria isolated from camel mastitis

Isolates	No. tested	K 30µg	OT 30µg	C 30µg	T 30µg	GM 30µg	S 10µg	PG 10 U	AP 10µg	E 5µg	NA 30µg
S. aureus	35	100.0	97.1	100.0	100.0	100.0	100.0	91.4	94.3	68.6	0.0
S. epidermidis	35	97.1	71.4	100.0	74.3	100.0	85.7	54.3	42.9	77.1	0.0
S. hyicus	35	100.0	100.0	100.0	100.0	100.0	100.0	97.1	97.1	88.6	25.7
S. intermedius	5	100.0	100.0	100.0	100.0	100.0	100.0	80.0	80.0	100.0	0.0
Micrococcus spp.	2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0
S. agalactiae	10	10.0	100.0	90.0	100.0	30.0	10.0	80.0	50.0	60.0	0.0
S. uberis	14	50.0	78.6	85.7	71.4	92.9	50.0	57.1	14.3	42.9	14.3
S. pyogenes	5	40.0	80.0	80.0	80.0	60.0	20.0	60.0	60.0	40.0	20.0
S. pneumoniae	2	50.0	100.0	100.0	100.0	100.0	50.0	50.0	50.0	50.0	0.0
Enterococcus faecalis	2	100.0	100.0	100.0	100.0	100.0	100.0	50.0	100.0	50.0	0.0
Past. haemolytica	8	87.5	12.5	25.0	12.5	100.0	37.5	0.0	0.0	0.0	12.5
C. bovis	8	50.0	50.0	87.5	50.0	100.0	50.0	25.0	25.0	12.5	0.0
A. pyogenes	3	66.7	100.0	100.0	100.0	100.0	66.7	100.0	100.0	100.0	0.0
B. cereus	10	80.0	80.0	100.0	70.0	90.0	80.0	20.0	20.0	50.0	50.0
E. coli	19	100.0	84.2	94.7	78.9	100.0	47.4	21.1	84.2	21.1	42.1
Enterobacter aerogenes	10	80.0	30.0	60.0	30.0	90.0	40.0	10.0	10.0	0.0	90.0
Klebsiella pneumoniae	5	80.0	80.0	100.0	40.0	100.0	80.0	0.0	20.0	0.0	80.0
Serratia marcescens	3	66.7	66.7	100.0	66.7	66.7	33.3	0.0	33.3	0.0	0.0
Aeromonas hydrophila	5	100.0	100.0	60.0	20.0	100.0	40.0	0.0	0.0	0.0	40.0
Proteus mirabilis	3	100.0	100.0	100.0	66.7	100.0	100.0	66.7	66.7	66.7	33.3

*: the results in percentage and for all regions

K = Kanamycin OT = Oxytetracycline C = Cloramphenicol

T = Tetracycline GM = Gentamicin S = Streptomycin

PG = Penicillin G AP = Ampicillin E = Erythromycin

NA = Nalidixic acid

µg = Milligram U: units

5. DISCUSSION AND CONCLUSIONS

The results of the questionnaire survey revealed that out of 20 camel owners interviewed in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region), 95% of them reported that milk and meat production and source of income were the main purposes of keeping camels. Schwartz and Dioli (1992) reported that camels play major roles in the economy and survival of the nomads in the arid and semi-arid zones of the east Africa. Teka (1991) reported that camel milk is the main food in the pastoral production system of Ethiopia.

The results of this study showed that 95% of the owners confirmed the presence of udder infection in lactating camels. However, 65% and 20% of the owners did not know the treatments and the causes of udder infections, respectively. Results of studies conducted in Ethiopia by Almaw and Molla (2000), Salah (2000), and Bekele and Molla (2001) confirmed the presence of camel mastitis in eastern, south western, and north eastern parts of the country. They also explained that the nomads are not normally aware of udder infections compared to other disease situations that negatively impact on the camel productivity. Questionnaire responses indicated that the milkers did not clean the udder nor did they wash their hands before milking. These factors have been associated with outbreaks of mastitis caused by environmental pathogens (Radostits *et al.*, 2000).

In Negele (Borena Region), only five cases (1.1%) of clinical mastitis were observed. This result is not significantly different from that reported by Almaw and Molla (2000) of 2.1% clinical mastitis of the dromedary camels in eastern Ethiopia. However, this proportion of clinical mastitis was lower than those reported by Bekele and Molla (2001) in Ethiopia, Barbour *et al.* (1985) in Saudia Arabia, Obied *et al* (1996) in Sudan of 12.5%, 15%, and 19.5%, respectively.

High prevalence of sub-clinical mastitis in the camel was recorded in Dire Dawa (46.2%) followed by Gewane (Afar Region) (45.4%) and Negele (Borena Region) (37.1%). Incontrast, these prevalences obtained in this study were higher than those recorded by Almaw and Molla (2001) and Bekele and Molla (2001) of 22.05% and 18.95% in eastern Ethiopia and 19.1% and 17.8% in north-eastern Ethiopia based on the CMT and bacteriological results, respectively.

The bacteriological results showed that *S. aureus* and coagulase-negative Staphylococci (*S. hyicus* and *S. epidermidis*) were the common microorganisms isolated from sub-clinical mastitis. These microorganisms have been diagnosed as the main causative agents of sub-clinical mastitis in camels (Abdurahman *et al.* 1995, Obied *et al.*, 1996; Bekele and Molla, 2001). But research conducted on bovine mastitis (Radostits *et al.*, 2000), coagulase-negative Staphylococci (CNS) are commonly isolated from normal milk samples, teat canals, and teat skin. In this species, they are regarded as opportunists in the causation of mastitis. *Streptococcus uberis*, *Streptococcus agalactiae*, and *Corynebacterium bovis* were also prevalent pathogens isolated from camel milk of sub-clinical mastitis. Similarly, Bekele and Molla (2001), El-Jakee (1998), Almaw and Molla (2000), and Abdurahman *et al.* (1995) have isolated these microorganisms from mastitic camels. Radostits *et al.* (2000) explained that *Streptococcus agalactiae* is a highly contagious obligate microorganism of the bovine and a major cause of udder infection. While, Todhunter *et al.* (1995) reported that the proportion of intra-mammary infections caused by environmental Streptococci such as *Streptococcus uberis* has markedly increased. In addition, these authors indicated that this pathogen is the leading cause of both sub-clinical and clinical mastitis in dairy cattle worldwide. *C. bovis* was considered as a minor pathogen. It is mildly pathogenic and the main reservoir is the infected glands or teat ducts (Radostits *et al.*, 2000).

In this study, *E. coli* was isolated from both sub-clinical and clinical mastitis. Radostits *et al.* (2000) reported that in contrast to contagious mastitis, environmental mastitis caused by coliform bacteria for example *E. coli*, is primarily associated with clinical mastitis, rather than sub-clinical mastitis. However, Saad and Thabet (1993) and Bekele and Molla (2001) isolated *E. coli* from mastitic camels and regarded as the principle agent for both clinical and sub-clinical mastitis in dromedary camels. The other colifoms isolated in this study were *Enterobacter aerogenes* and *Klebsiella pneumoniae*. The important role of *Klebsiella pneumoniae* in camel mastitis has been reported in Egypt (El-Jakee, 1998).

Further microorganisms isolated from sub-clinical mastitic milk were *Pasteurella haemolytica* and *Bacillus cereus*. These results are in agreement with those of Bekele and Molla (2001) who isolated *Pasteurella haemolytica* and *Bacillus spp.* from sub-clinical mastitis in camels in north-eastern Ethiopia. However, Radostits *et al.* (2000) considered these bacteria as uncommon pathogens that cause sporadic and severe mastitis. In addition, they usually affect one animal in the herds.

The microorganisms isolated from clinical cases in this study were *Staphylococcus aureus*, *Streptococcus uberis*, *S. hyicus*, *Bacillus cereus*, *E. coli*, and *S. epidermidis*. Similar isolates have been reported by different investigators as important agents of clinical mastitis in camels (Quandil and Qudar, 1984; Ramadan *et al.*, 1987; Al-ani and Al-Shareefi, 1997; Almaw and Molla, 2000; Bekele and Molla, 2001).

Of quarter milk samples examined, 14.26% of them were positive in the CMT. Kappa statistic indicated a slight agreement between CMT and bacteriological groupings (MAP, MIP, and NI). However, a significant positive correlation was found between the two tests. These observations are similar to that of Bekele and Molla (2001) who reported a strong positive correlation between CMT scores and bacteriological results in their study on Afar milking camels. Furthermore, Younan *et al.* (2001) recorded that the CMT can be applied as screening test for the detection of intra-mammary infection with *S. aureus* and *Streptococcus agalactiae* in the camels in Kenya. In the current study, the results have also shown that most of the positive cases detected by CMT were associated with the major pathogens. This finding has previously been reported in Sudan by Abdurahman *et al* (1995). The same author has reported that quarter milk samples infected with major pathogens had higher values for CMT, SCC, and ATP than those quarters infected with minor pathogens as well as non-infected quarters of dromedary camels.

Significantly close correlation between CMT scores and SCC was observed although the kappa statistic indicated a slight agreement between the two tests. Furthermore, the comparison of log SCC of the CMT scores showed that the difference among the medians log SCC of CMT scores was statistically significant. These findings are in agreement with results reported by Radostits *et al.* (2000) for bovine mastitis and by Abdurahman (1996) for camel mastitis. They observed that both SCC and CMT were useful in predicting the infection status of the udder.

Both log SCC and SCC cut-off level 2.5 x 10^5 ml^{-1} showed slight agreement when compared with bacteriological groupings (MAP, MIP, and NI). Nevertheless the differences were not statistically significant. This could be due to the fact that *E. coli* and other coliforms can multiply rapidly in infected quarters with low cells (Quinn *et al.*, 1994). Radostits *et al.* (2000) indicated that SCC rises during the first 2 days after udder infection and returns to normal over the next 10 days. Recently, Salah (2000) explained that high SCC in non-infected quarters could be due to the lesions on the teats and udder skin of the camels. The same author also suggested that high

SCC could be observed even after the elimination of the infection. Moreover, in the present study the quarter milk samples were frozen at - 20°C, this might have affected the SCC results.

The SCC cut-off level 2.5 x 10^5 ml^{-1} showed low agreements among the three stages of lactation (Table 9). Large numbers of cell fragments were observed in camel milk by Abdurahman (1992). Similar cell fragments were found in goat milk but without reaction with the CMT (Schalm *et al.*, 1971). Furthermore, Kospakov (1976) reported that camels free of infection had a higher SCC than cow. This may explain the poor agreement between SCC cut-off level 5 x 10^5 ml^{-1} and the different age groups in this study. The result that parity of 5 - 6 could be a risk factor is in agreement with that of Kospakov (1976). The same author reported that SCC of the camel increases as lactation progresses and can be compared to that described in the cow (Schalm *et al.*, 1971).

Results of the risk factors analysis showed that the use of anti-suckling devices was a contribution factor for camel mastitis. It is probable that these devices positively enhanced the spread of intra-mammary infection among camels. Similarly, in Sudan Abdurahman *et al.* (1995) and Obied *et al.* (1996) explained that the use of anti-suckling devices might be regarded as predisposing factor for camel mastitis.

The results of tick infestations and teat lesions are in contrast with the observations of Bekele and Molla (2001). These researchers suggested that heavy tick infestation and teat lesions might be responsible for udder infection and also lead to udder abnormalities and deformities and blind teats in Afar milking camels. The disagreement can be attributed to the large number of the camels that were observed having tick infestations and a few of them with teat lesions in this study. These could have affected the outcome of the final analytical results..

The results of in-vitro anti-microbial susceptibility test revealed that oxytetracycline, tetracycline, and cloramphenicol were effective drugs against camel mastitis pathogens with exception of *Pasteurella haemolytica* and *Enterobacter aerogenes*. These findings are in agreement with those of Salah (2000). A high level of resistance of mastitis pathogens of camels was recorded against nalidixic acid and erythromycin. It was noticed that many types of antibiotics have been used for treatment of infectious diseases in camels for long times (Salah, 2000; Younan *et al.*, 2001). This

may explain the increasing of resistance patterns of some mastitis pathogens of camel against some of the commonly used anti-microbial agents

In conclusion, the results of the present study revealed that mastitis in camel is prevalent in Negele (Borena Region), Dire Dawa, and Gewane (Afar Region). California Mastitis Test (CMT) can be applied for the detection of the udder infection in camels. The pathogenic bacteria that cause mastitis in camels are similar to those of the cows. Use of anti-suckling devices was incriminated as a risk factor in spreading of intra-mammary infection in camels.

Based on the results of the present study the following are recommended:

- The Somatic cell counts (SSC) of the normal and mastitic camel milk requires further investigations.

- An attempt should be made to increase awareness of camel owners on the importance and impact of udder infection on public health and milk yield.

- In order to control and prevent mastitis in camels, it will be valuable to avoid risk factors of camel mastitis such as use of anti-suckling devices.

- Monitoring of zoonotic diseases such as salmonellosis, brucellosis and tuberculosis in the camel milk herds of Ethiopia is required.

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Appendix 1: Sample Collection Form

Date of sample collection ---------- Sample code ----------

Owner's name ----------

Address: ----------

Animal identification: Age---------- Calving date---------- Parity----------

◆ Previous History of udder problem:

Yes---------- No ----------

◆ Physical examinations of the udder:

Conformation: even ---------- uneven----------

Teat lesion: present---------- absent----------

Tick infestation: present---------- absent----------

Gross milk quality:

watery---------- blood tinged---------- clots/flakes---------- normal----------

◆ CMT score (0, T, 1, 2, 3):

RR---------- RF---------- LF---------- LR----------

◆ Form of mastitis:

Chronic---------- Clinical---------- Sub-clinical ----------

◆ Remarks:

Appendix 2: General Questionnaire

Date---------- Code No. ----------

Owner's Name ----------

Address ----------

1. Herd Size

Number of Species owned

Sheep---------- Goats---------- Cattle ---------- Camels---------- Equine----------

2. purposes of keeping camels

---

3. The main constraints for camel rearing

Feed Shortage---------- Water Scarcity---------- Diseases----------

4. Presence of mastitis

Yes---------- No----------

5. Cause of camel mastitis

---

6. Type of treatment of camel mastitis

---

7. Application of cauterization for udder problems

Yes---------- No----------

8. Use of anti-suckling devices

Yes ---------- No----------

9. Tick control

Manual removal ---------- Use of Acaricides---------- Other-------- No control Practice---------

10. Frequency of milking

Once---------- Twice ---------- Three times---------- Four times----------

11. Cleaning the udder before milking

Yes ---------- No----------

12. Washing the hands before milking

Yes---------- No----------

Name of interviewer ----------

Signature----------

Appendix 3:

Diagnostic procedures applied for bacteriological isolation (Quinn *et al.*, 1994)

1. Biochemical reactions and other characteristics of *Staphylococci* and *Micrococci*

Species	Gm stain	Catalase	Coagulase	Oxidase.	O-F glucose	Haemolysis	Pigment Production	Maltose Fermented	Polymxin-B 300 Unit disk
S. aureus	+ ve cocci	+	+	-	F	+	+ /.	+	R
S. intermedius	+ ve cocci	+	+	-	F	+	-	(±)	S
S. hyicus	+ ve cocci	+	d	-	F	-	-	-	R
S. epidemidis	+ ve cocci	+	-	-	F	(d)	-	+	R
Micrococcus spp.	+ ve cocci	+	-	-	O	-	-	•	•

Gm. Stain: Gram-stain
+ ve: Positive
O-F: Oxidation-Fermentation
+: 90% or more strains positive
-: 90% or more strains negative
d: 11-89% strains positive
( ): delayed reaction
±: 90% or more strains weakly positive
•: not known
S: Susceptible
R: Resistant (zone of less than 10 mm)

2. Biochemical reactions and other characteristics of *Streptococci* and *Enterococci*

Species	Gm. Stain	Catalase	Oxidase	Coagulase	Haemolysis	Aesculin hydrolysis	Growth on MacConkey agar	CAMP	Sorbitol	Salicin
S. agalactiae	+ cocci	-	-	-	β (α,γ)	-	-	+	-	(+)
S. dysgalactiae	cocci	-	-	-	α (β,γ)	-	-	-	-	-
S. uberis	+ cocci	-	-	-	α (γ)	+	-	-	+	+
S. pyogenes	+ cocci	-	-	-	β	-	-	-	-	+
S. pneumoniae	+ cocci	-	-	-	α	-	-	-	-	V
Enterococcus faecalis	+ cocci	-	-	-	α (β,γ)	-	+	-	+	+

+: positive

-: negative

(+): majority of strains positive

V: variable reaction

3. Characteristics of *Corynebacterium*, *Actinomyces*, and *Bacillus*

Species	Gm. Stain	Catalase	Oxidase	Motility	Haemolysis	CAMP	Growth on 9% NaCl	Other Characteristics
Corynebacterium bovis	+ rods	+	-	-	-	-	+	Small, white, dry colonies. glucose +ve.
Actinomyces pyogenes	+ rods	-	-	-	+	+	-	Small colonies and hazy hae molysis.
Bacillus cereus	+ rods	+	-	+	+	•	•	Forms endospores, clear hae molysis, large and flat coloni

+: positive -: negative • : not known

4. Characteristics of Gram-negative and oxidase positive rods

Species	Gm. Stain	Catalase	Oxidase	Motility	Haemolysis	Growth on Mac-Conkey agar	Lactose	Other Characteristics
Aeromonas hydrophila	- rods	+	+	+	+	+	V	Colonies are large, flat and greyish, indole +ve.
Pasteurella haemolytica	- rods	V	+	-	+	+	(-)	No smell, indole –ve, Pin-poi colonies on MacConkey aga
Pasteurella multocida	- rods	+	+	-	-	-	(+)	Colonies with sweetish smel Indole +ve.

+: positive -: negative (+): most strains positive
(-): most strains negative V: strains vary

5. Biochemical reactions of Enterobacteriaceae

Species	Gm stain	Characteristic Colony	Oxidase	Motility (36°C)	Urease	Lysine decar-boxylase	IMViC	Acid form		
								Lactose	Sorbitol	Manitol
Escherichia coli	- rods	mucoid (rare)	-	(+)	-	(+)	+/+/-/-	+	+	+
Klebsiella pneumoniae	- rods	mucoid	-	-	+	+	-/(-)/+/+	+	+	+
Enterobacter aerogenes	- rods	mucoid	-	+	-	+	-/-/+/+	+	+	+
Citrobacter diversus	- rods	round and greyish	-	+	(+)	-	+/+/-/+	d	+	+
Serratia marcescens	- rods	red pigment at 37°C*	-	+	(-)	+	-/(-)/+/+	-	+	+
Proteus vulgaris	- rods	foul odour	-	+	+	-	+/+/-/(-)	-	-	-
Proteus mirabilis	- rods	foul odour	-	+	+	-	-/+/(-)/d	-	-	-

+: 90-100% strains positive -: 0-10% strains positive (+): 76-89% strains positive
(-): 11-25% strains positive d: 26-75% strains positive
IMViC: indole, methyl red, voges-proskauer, and citrate

8. CURRICULUM VITAE

PERSONAL Data

Name	Atif El amin Abdel Gadir
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1977 – 1986	El Izba (2) primary school El Ameria (1) intermediate school
1987 – 1990	Bahri high secondary school
1992 – 1996	Faculty of Veterinary Science University of Khartoum

Publications

1- Intisar, E., Atif, E., Mohmmed, O., Saud, M., Badria, B., Khitma, H. (1999): Prevalence of sleeping sickness in Sudan. Proceedings of the 25th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 27th Sep.-1st Oct. Mombasa, Kenya. Pp 107-110.

2- Atif, E., Intisar, E., Khalil, M., Mohmmed, O., Khitma, H., Saud, M., Kyule, M. (2001): Sero-epidemiological studies on sleeping sickness situation using CATT/CIATT in Khartoum state, Sudan. East African Medical Journal. (submitted for publication).

3- Atif, E., Intisar, E., Khitma, H., Khalil, M., Mubarak, M. (1999): Application of CATT and CIATT for diagnosis of animal trypanosomiasis. (unpublished).

Declaration sheet

I, the undersigned, declare that the thesis is my original work and has not been presented for degree in any University.

Name: Atif El amin Abdel Gadir

Signature.....

Date of submission: 23 November 2001

This thesis has been submitted for examination with our approval as University advisors

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Cross-sectional study of mastitis in c camels (Camelus dromedarius)in selected sites of Ethiopia

Atifel Amin

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