

Thesis Ref. No. \_\_\_\_\_

**CAMEL MASTITIS: PREVALENCE, RISK FACTORS, AND ISOLATION OF  
MAJOR BACTERIAL PATHOGENS IN JIGJIGA DISTRICT OF FAFAN ZONE,  
EASTERN ETHIOPIA**



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

**DEPARTMENT OF VETERINARY CLINICAL STUDIES**

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BISHOFTU, ETHIOPIA**

**CAMEL MASTITIS: PREVALENCE, RISK FACTORS, AND ISOLATION OF  
MAJOR BACTERIAL PATHOGENS IN JIGJIGA DISTRICT OF FAFAN ZONE,  
EASTERN ETHIOPIA**



**A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis  
Ababa University in partial fulfillment of the requirements for the Degree of Master  
of Veterinary Science in Veterinary Epidemiology**

**By  
Muluaem Kidane**

**June, 2023  
Bishoftu, Ethiopia**

Addis Ababa University  
College of Veterinary Medicine and Agriculture  
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## **ABBREVIATIONS**

CAMP	Christie Atkins Munch Peterson
CMT	California Mastitis Test
EC	Electrical Conductivity
EMB	Eosin-Methylene Blue
LDH	Lactate Dehydrogenase
MR	Methyl-Red
MSCC	Microscopic Somatic Cell Count
NAGase	N-acetyl- $\beta$ -D-glucosaminidase
SCC	Somatic Cell Count
SFMT	Surf Field Mastitis Test
SLST	Sodium Lauryl Sulfate Test
Spp.	Species
TSIA	Triple Sugar Iron Agar
VP	Voges-Proskauer
WST	White Side Test

## ABSTRACT

A cross-sectional study was conducted from December 2022 to April 2023 to determine the prevalence, associated risk factors, and major bacterial pathogens causing mastitis in lactating camels in Jigjiga District of Fafan Zone, Eastern Ethiopia. A total of 324 lactating camels were randomly selected and examined for clinical and subclinical mastitis using the California Mastitis Test (CMT). The overall prevalence of camel mastitis was 33.6%,  $n=109$ , (95% CI: 28.5%-39.1%), with clinical mastitis of 6.5%,  $n=21$ , (95% CI: 4.0%-9.7%) and subclinical mastitis of 27.1%,  $n=88$ , (95% CI: 22.4%-32.3%). Out of 1296 teat quarters tested for mastitis, 23.8%,  $n=309$ , were found to be positive. Among the total affected quarters, 1.5%,  $n=19$ , and 22.7%,  $n=290$  teats were found blind and CMT positive, respectively. Among the putative risk factors assessed in this study, age, body condition score, and use of anti-suckling devices were significantly associated ( $p<0.05$ ) with the occurrence of camel mastitis. The odds of mastitis occurrence were nearly four times (AOR: 3.40) (95% CI =1.61–7.17,  $P=0.001$ ) and 4.20 (95% CI =1.97-8.94,  $P =0.000$ ) higher for medium and poor body condition scores, respectively, as compared to a good body condition score. Adult camels were nearly five times (AOR: 5.27; 95% CI=2.81–9.90,  $P=0.000$ ) more likely to be infected than their young counterparts. Mid lactation (AOR: 0.22, 95% CI: 0.10–0.45,  $P = 0.000$ ) and late lactation (AOR: 0.23, 95% CI: 0.11–0.48,  $P=0.000$ ) were statistically found to be protective as compared to the early lactation stage. Likewise, utilization of an anti-suckling device exhibited a significant association (AOR: 10.08; 95% CI=5.16–19.69,  $p =0.000$ ) with the prevalence of mastitis. Among the 290 quarter milk samples that tested positive for CMT, 109 were subjected to bacteriological analysis. Of these, 89% (97/109) yielded mastitis bacteria, whereas 11% (12/109) displayed no growth. Of the total bacterial isolates, *coagulase-negative Staphylococci* (26.8%;26/97) were the most frequent bacterial isolates, whereas *Streptococcus agalactiae* (5.1%;5/97) was the least isolated bacterial pathogen. From the 34 households owning camels interviewed, 88.2% (30/34) of them responded that they were aware of clinical mastitis, whereas all were not aware of subclinical mastitis. The current study revealed a high prevalence of camel mastitis linked to several risk factors. Prevention and control strategies should be implemented to reduce the prevalence of camel mastitis, including regular cleaning of the udder, milking camels with mastitis at the end, timely treatment, and not using anti-suckling devices.

**Key Words:** - *Bacterial, Camel-mastitis, Ethiopia, Milk, Prevalence, Quarter*

## 1. INTRODUCTION

The one humped camel (*Camelus dromedaries*), is the most widespread and widely distributed animal on the tropical and subtropical continents of Africa and Asia. It makes a substantial contribution to resource utilization and human survival in dry and arid regions (Younan and Abdurahman, 2004). The camel is a multipurpose animal kept for milk, meat production, and transportation. It is also a financial reserve for pastoralists and plays an important role in social prestige and wealth (Ramet, 2001).

Of the more than 35 million camels in the world (Faye, 2020), the camel population in Ethiopia is estimated at 4.8 million, and the one-humped camel is the only type of camel found in Ethiopia (Mirkena *et al.*, 2018). Camels are predominantly reared in the drier areas of Ethiopia such as Afar, Ethiopian Somali and the eastern and southern parts of Oromia region (Kena, 2022).

In arid and semiarid regions of Africa and Asia, camel pastoralists rely heavily on camel milk as a staple food because it is more nourishing, easily digestible, and can be preserved for a longer period of time than other types of milk (Abera *et al.*, 2010; Abera *et al.*, 2016). Protein, lipids, minerals, and vitamins, particularly vitamin C, are all abundant in camel's milk. The high vitamin C concentration is crucial to the human diet, especially in dry regions where green produce and fruit are hard to come by (Abera *et al.*, 2010). It is also lower in fat and lactose than cow's milk, making it a good alternative for people who are lactose intolerant (Faye, 2020).

Despite the importance of their milk, dromedary camels are susceptible to mastitis, a complex disease that affects dairy animals worldwide and causes significant economic losses due to both clinical and subclinical mastitis, the latter of which necessitates indirect methods of diagnosis (Matofari *et al.*, 2003). More than 25% of lactating camel are affected by mastitis (Abdurahman and Younan, 2002; Abera *et al.*, 2010) and cause approximately 70% losses in milk production (Alamin *et al.*, 2013).

Mastitis is divided into two categories: clinical and subclinical, depending on the level of inflammation, and, is characterized as contagious or environmental, depending on the source of organisms involved (Quinn *et al.*, 2004). Contagious mastitis is caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis*. Environmental mastitis is brought on by bacteria that can be found in manure, bedding,

water, soil, and calving pads. These bacteria include *Streptococcus uberis*, *Streptococcus dysgalactiae*, coliforms like *Escherichia coli*, and *Klebsiella*, to name a few (Khan and Khan, 2006).

The prevalence and causes of mastitis differ markedly due to individual herd management (Regassa *et al.*, 2013). An important risk factor for mastitis in camels is the lack of milking hygiene practices such as the washing of hands before milking, washing of udders before and after milking, and milking camels with healthy udders before milking camels with mastitis (Ahmad *et al.*, 2011).

Teat invasion occurs most frequently during milking. Organisms from the manure, bedding, etc. or those found on injured skin at the tip of the teat might easily enter into an opened or partially opened teat canal (Michel, 2000). Once a microorganism attains the gland, it can multiply and begin generating toxins; however, the gland will begin to stimulate the discharge of inflammatory mediators to draw phagocytes to clear the pathogens; inflammation of the mammary gland occurs (Bařłowska *et al.*, 2011).

Clinical mastitis manifests as redness, heat, swelling, discomfort, blood clots, or discolored milk, which are five general indications of mastitis. Subclinical mastitis in camels is difficult to detect because it does not cause visible symptoms in the udder or milk (Seligsohn *et al.*, 2021). It is characterized by apparently normal milk and increased leukocyte counts (Geresu *et al.*, 2021). Chronic mastitis is an inflammatory illness that can last for months and persist from lactation to lactation (Radostits *et al.*, 2007).

Clinical mastitis can be identified by looking for clotted milk, but sub-clinical mastitis can only be proven using a variety of tests, including the Microscopic Somatic Cell Count (MSCC) (Sharma *et al.*, 2010; Hoque *et al.*, 2014), Electrical Conductivity (EC), and the California Mastitis Test (CMT), as well as the Whiteside and Surf Field Mastitis Tests. Additionally, enzymatic assays such as colorimetric and fluorometric assays have been created (Viguier, 2009).

Camel mastitis is a significant problem in Ethiopia, with both clinical and subclinical mastitis being prevalent in traditionally managed camels (Abera *et al.*, 2010). The economic importance of mastitis includes loss of milk production and quality, reduction in milk price due to high somatic cell count, and increased cost of treatment (Jilo *et al.*, 2017). According to the evidence, subclinical mastitis harms the animal, reduces milk

production, changes the characteristics of the milk, hinders preservation and processing, and poses a public health risk to those who drink camel milk (Tibary *et al.*, 2000; Gramay and Ftiwi, 2018). The socio-economic burden of mastitis in camel includes the cost of veterinary services, the cost of discarded milk plus milk production loss, and the cost of drugs (Ranjan *et al.*, 2022). Control and prevention can be achieved by ensuring that teats are kept dry, using liner slips between teats whenever possible, utilizing effective germicidal solutions for teat dips, and consistently monitoring the milking process (Schroeder, 2012).

In recent times, the occurrence of mastitis among lactating camels has become a growing concern in various camel-rearing countries, including Somalia (Mohamud *et al.*, 2020), Sudan (Alamin *et al.*, 2013), Kenya (Kashongwe *et al.*, 2017), United Arab Emirates (Aqib *et al.*, 2022), and different parts of Ethiopia (Husein *et al.*, 2013; Abera *et al.*, 2016; Ahmed *et al.*, 2019). However, there are few previous works on prevalence of camel mastitis, related risk factors, and causes of the major bacterial pathogens in Jigjiga District, Eastern Ethiopia (Abera *et al.*, 2010; Husein *et al.*, 2013). In light of the essential role that camel milk plays in the livelihood of many people in the Jigjiga District, Eastern Ethiopia, it is imperative access to up-to-date information on the nature of mastitis and the major bacterial pathogens associated with its occurrence of Camel mastitis. Therefore, the research objective emphasis:

General objective:

- To determine the prevalence of camel mastitis in the study area
- To identify the possible risk factors associated with camel mastitis
- To isolate the major aerobic bacterial pathogens from camel mastitis

Specific objectives:

- To estimate the prevalence of clinical and subclinical camel mastitis both at camel level and quarter level
- To identify the potential risk factors of camel related and milking procedure which can be associated with camel mastitis
- To isolate both gram positive and gram negative bacterial that causes camel mastitis

## **2. LITERATURE REVIEW**

### **2.1. Camel Distribution and Utilization in Ethiopia**

Of the more than 35 million camels in the world (Faye, 2020), the camel population in Ethiopia is estimated at 4.8 million, and the one-humped camel is the only type of camel found in Ethiopia (Mirkena *et al.*, 2018, Faye, 2020). Camels are predominantly kept in the pastoral and agro-pastoral production systems in Ethiopia (Zelege, 2017). More than 50% of Ethiopia's pastoralist area is occupied by pastoralists from the Borana, Kereyu, Afar, and Somali tribes (Husein *et al.*, 2013; Abera *et al.*, 2016). These areas have harsh conditions of arid or semi-arid rangelands and are the dominant mode of livelihood in the country (Mirkena *et al.*, 2018).

Camel milk is an essential food for pastoralists because it is more nourishing, thirst-quenching, easily digestible, and can be preserved for a longer period of time than other types of milk (Abera *et al.*, 2010; Abera *et al.*, 2016). Protein, lipids, minerals, and vitamins, particularly vitamin C, are all abundant in camel's milk. The high vitamin C concentration is crucial to the human diet, especially in dry regions where green produce and fruit are hard to come by (Abera *et al.*, 2010).

### **2.2. Camel Mastitis**

Mastitis is an inflammatory response of the udder tissue in camels that can cause swelling, heat, and pain in the mammary gland and the milk may become discolored and clotted (Tigani-Asil *et al.*, 2020). There are two types of mastitis in camels: Clinical mastitis: This type of mastitis is characterized by redness, swelling, and hardening of the udder and a failure to produce milk (Tigani-Asil *et al.*, 2020). Subclinical mastitis: This type of mastitis is not easily detected as there are no obvious signs of inflammation or abnormality in the milk, but it can lead to reduced milk yield (Geresu *et al.*, 2021).

Infectious agents, hosts, and environmental factors determine the occurrence of camel mastitis (Radostits *et al.*, 2007; FAO, 2014). Compared to cattle, camels have a relatively low incidence of mastitis; however, due to hand milking and teat deformity in dairy camels, the condition may become more common (Al-Tofaily and Alrodhan, 2011).

### 2.2.1. Etiology

Mastitis can be divided into two categories: clinical and subclinical, depending on the level of inflammation, and can be characterized as contagious or environmental, depending on the organisms involved (Quinn *et al.*, 2004).

The causative agents of camel mastitis are not well-defined and studied (Seifu and Tafesse, 2010). However, a few available works of literature indicate that the major bacterial pathogens isolated from subclinical mastitis are *Staphylococcus*, *Escherichia coli*, *Corynebacterium*, *Streptococcus*, *Bacillus*, and *Micrococcus species* (Mengistu *et al.*, 2010; Hamad, 2012). Clinical mastitis is mostly caused by bacteria, with the most important causative agents being *S. aureus*, *Strep. uberis*, *dysagalactia*, *E. coli*, and *Klebsiella spp.* (Iyer *et al.*, 2014).

Depending on the organisms involved, pathogens that cause contagious mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Uberis*, whereas *Streptococcus uberis*, *Streptococcus dysgalactiae*, coliforms like *Escherichia coli*, and *Klebsiella* are brought on by the environment (Khan and Khan, 2006).

### 2.2.2. Epidemiology

The incidence and causes of mastitis vary considerably due to individual herd management (Regassa *et al.*, 2013). The prevalence of mastitis in camels varies from country to country, with the mean prevalence of mastitis being 45.66% of the global camel population (Aqib *et al.*, 2022). The prevalence of camel mastitis varies by region and study. For example, a study in southern Ethiopia showed an overall incidence of 22.4%, with 4.3% clinical and 18.1% laboratory-confirmed (Geresu *et al.*, 2021).

### 2.2.3. Risk Factors associated with camel mastitis

An essential threat aspect for mastitis in camels is the dearth of milking hygiene practices, including the washing of fingers earlier than milking, washing of udders earlier than and after milking, and milking camels with wholesome udders earlier than milking camels with mastitis (Ahmad *et al.*, 2011). In pastoralist camel herds, such hygiene practices are very uncommon (Kashongwe *et al.*, 2017). Other factors related to an increased occurrence of mastitis in camels are growing age, an expanded variety of parities, the presence of lesions

at the udder, the presence of tick infestations of the udder, and the usage of conventional teat tying to prevent the calf from suckling (Abera *et al.*, 2010; Regassa *et al.*, 2013).

The maximum prone intervals for contamination with environmental pathogens are within the dry duration, particularly all through the primary weeks and early lactation of the remaining 10 days earlier than calving. Incidence rates are twice as high during calving as they are during the drying-off period. Mastitis as a whole is a complicated health issue and serves as a typical illustration of how microbes, host factors, and the environment interact (Khan and Khan, 2006).

#### 2.2.4. *Transmission and mode of infection*

Mastitis can be spread from one animal to another during the milking process, and camels can serve as a major reservoir of the pathogens (Rahmeh *et al.*, 2022). The transmission mechanisms depend on the bulk of the infection in the environment, including infected quarters, the efficiency of milking personnel, and the susceptibility of the animal/camel, which is related to the stage of lactation, age of the camel (older animals more susceptible), and level of inherited immunity (Jilo *et al.*, 2017). Skin lesions on the teats or udder also identified as risk factors for the transmission of mastitis in pastoralist dairy camel herds (Seligsohn *et al.*, 2021).

Contagious mastitis pathogens can be discovered on an animal's skin or in its udders. Teat-cup-to-teat cup cross-contamination, contaminated milk splashes and sprays during stripping, and the person milking the animal's hands all contributed to their spread. Environmental mastitis is caused by bacteria that can be found in manure, bedding, water, soil, and calving pads. Environmental pathogen does not live in the milk, although contagious mastitis pathogens can occasionally persist and spread during the milking process (Khan and Khan, 2006).

#### 2.2.5. *Pathogenesis*

Teat invasion occurs most frequently when milking. Organisms that are already in the milk or at the teat end are discharged into the teat canal and cistern when unwanted air is let into the milking machine. The teat canal is still dilated one to two hours after milking; however, a damaged teat canal may continue to be partially open for an endless amount of time.

Organisms from the surrounding region (manure, bedding, etc.) or those found on injured skin at the tip of the teat might easily enter an open or partially open canal (Michel, 2000).

Once a microorganism attains the gland, it can multiply and begin generating toxins; however, the gland will begin to stimulate the discharge of inflammatory mediators to draw phagocytes to clear the pathogens. This irritation cause harm to gland of alveoli that ultimately lose the shape. When the blood-milk barrier is broken, then additives from the extracellular fluid will enter the gland, such as sodium, chloride, hydrogen, potassium, and hydroxide ions. Once those factors enter the gland, they may blend with milk, which can additionally encompass blood if the harm is severe. At this stage, visible symptoms will be discovered at the udder, which include swelling and redness, in addition to the milk's color, pH, water content, and presence of flakes and clots (Seifu and Tafesse, 2010; Barłowska *et al.*, 2011).

#### 2.2.6. *Symptoms and Signs*

Different signs and symptoms apply depending on whether the mastitis is clinical, subclinical, or even chronic. Clinical mastitis is characterized by five overt signs of mammary inflammation: redness, heat, swelling, pain, and clotting or discoloration of the milk. The two main symptoms are loss of appetite and an elevated body temperature ( $> 39.5^{\circ} \text{C}$ ) (Radostits *et al.*, 2007). Generalized and systemic signs such as fever, anorexia, and depression may also be observed (Jilo *et al.*, 2017).

Subclinical mastitis in camels is difficult to detect because it does not cause visible symptoms in the udder or milk (Seligsohn *et al.*, 2021). It is characterized by apparently normal milk and increased leukocyte counts (Geresu *et al.*, 2021). Other indicators of subclinical mastitis are: reduced milk yield, presence of bacterial pathogens and positive mastitis card test (Alhafiz *et al.*, 2022).

Chronic mastitis is an inflammatory process that exists for months and may persist from one lactation period to another. It exists for the maximum element as sub-scientific and might flare up periodically as a sub-acute or acute shape for a quick length of time. The results are hard lumps in the udder from the "walling off" of bacteria and the formation of connective tissue (Radostits *et al.*, 2007).

### 2.2.7. *Diagnosis*

The diagnosis of camel mastitis can be done using various methods, including clinical and subclinical examinations. Clinical examination: Clinical mastitis can be diagnosed by observing the udder, which may be swollen, painful, and hot to the touch. The milk may also contain blood, pus, or other abnormalities (Jilo *et al.*, 2017).

Subclinical mastitis can be diagnosed by testing the milk for somatic cell count (SCC), lactate dehydrogenase (LDH) activity, and N-acetyl-D-glucosaminidase (NAGase) activity. SCC is the standard method for detecting subclinical mastitis in cows, and it has been investigated at the quarter level in camel milk. NAGase and LDH activity are useful as diagnostic markers in cows, and they have also been tested in camels (Seligsohn *et al.*, 2021). Other tests include the Whiteside test (WST), the Surf field mastitis test (SFMT), the sodium lauryl sulfate test (SLST), the microscopic somatic cell count (MSCC) (Hoque *et al.*, 2014; Sharma *et al.*, 2010), and the electrical conductivity (EC) (Hegde *et al.*, 2013). Additionally, enzymatic assays such as colorimetric and fluorometric assays have been created (Viguier, 2009).

### 2.2.8. *Treatment*

Although daily intra-mammary infusion using antibiotic formulations used in cattle has been suggested in certain publications, there has been criticism of this strategy because of the unique anatomy of the camelid udder and because it is challenging to deliver such treatment (Saleh and Faye, 2011).

Systemic antibiotics and anti-inflammatory medications are used to treat acute mastitis, along with routine removal of the mammary glands. The local edema can be reduced with hydrotherapy. Three distinct teat canals that open into the teat sphincter can occasionally be found in the camel udder's teat. Different gland complexes are drained by various channels (Sanaa, 2005).

### 2.2.9. *Economic Importance and Public Health Significance*

Camel mastitis has both economic and public health significance (Hegazy *et al.*, 2004; Al-Majali *et al.*, 2008). Mastitis causes a loss of milk production and quality, resulting in a reduction in milk price due to a high somatic cell count (Jilo *et al.*, 2017). The economic impact of clinical mastitis in dromedary camels includes milk production loss and the cost

of veterinary services (Ranjan *et al.*, 2022). Subclinical mastitis in camels can lead to significant economic losses and reduced milk yield, and quality, affecting household economies (Seligsohn *et al.*, 2021). In general, mastitis has been estimated to affect more than 25% of lactating she-camels (Saleh and Faye, 2011; Alamin *et al.*, 2013). Mastitis is also known to cause approximately 70% losses in milk production (Fazhani *et al.*, 2011).

Consuming milk from camels with mastitis can cause a potential health risk to humans and expose them to harmful bacteria and fungi. The bacterial organisms that can transmit from mastitic camel milk to humans are *Staphylococcus aureus*, *Streptococcus spp*, *Micrococcus spp*, *Streptococcus agalactiae*, *Coagulase negative staphylococci*, *Staphylococcus epidermis*, *Pasteurella haemolytica*, and *Escherichia coli* (Aqib *et al.*, 2022). Fungal organisms that cause camel mastitis and can transmit to human are the genera *Penicillium*, *Candida*, *Phanerochaete*, *Cladosporium*, and *Aspergillus* (Rahmeh *et al.*, 2022). Therefore, it is essential to ensure the quality of camel milk before consumption to avoid any potential health risks (Geresu *et al.*, 2021; Seligsohn *et al.*, 2021; Ranjan *et al.*, 2022).

#### 2.2.10. Control and Prevention

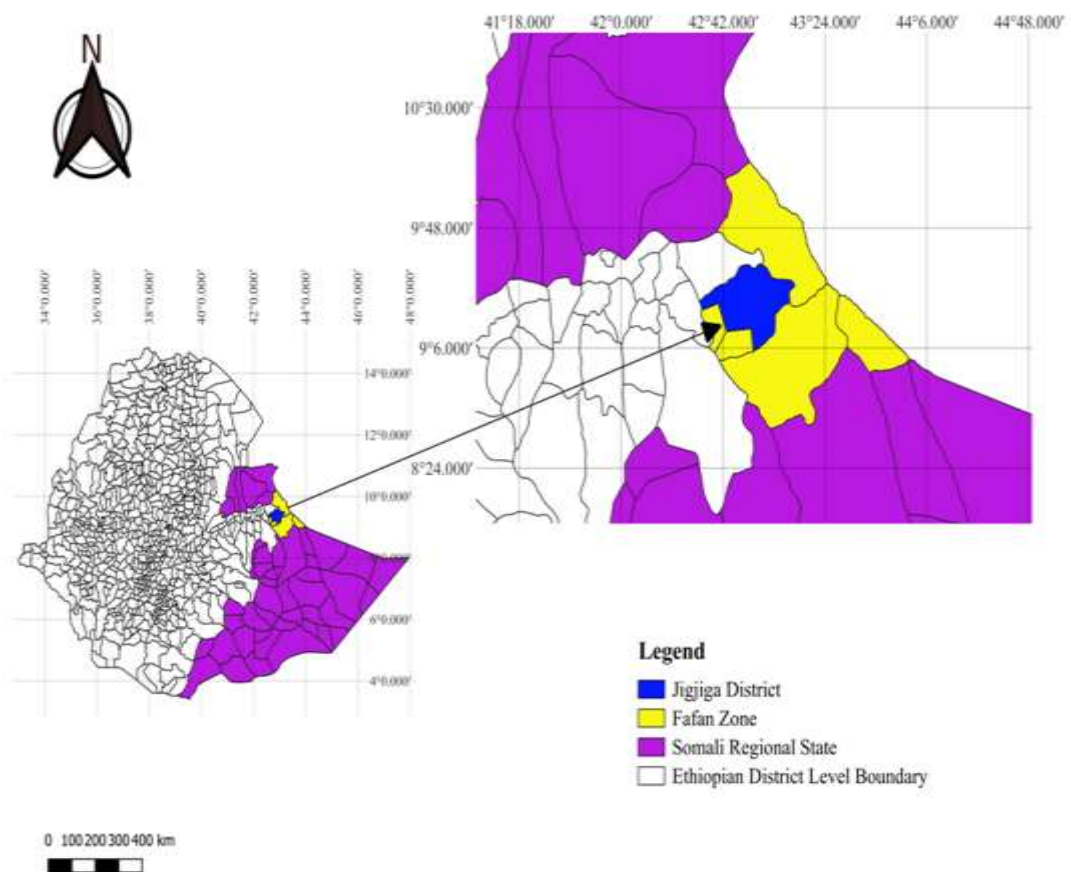
The important points to keep in mind while controlling mastitis are that prevention is the number one procedure through ensuring dry teats, the presence of liner slips between teats as much as possible, teat dips in effective germicidal solution, and always observing the milking procedure (Schroeder, 2012).

Mastitis can be prevented or decreased by increasing animal health and udder hygiene; however, camel keepers do not currently appear to be using any modern mastitis control methods. Even when the camel is dry, care must be taken to maintain the health and hygiene of the udder. Herbal medicine is still utilized as both traditional preventive and curative medicine in several nations, such as Ethiopia (Regassa *et al.*, 2013). Experienced camel owners use traditional folk remedies to treat mastitis, including various extracts from the roots, leaves, seeds, and exudates of different plants, as well as branding with hot iron (Geresu *et al.*, 2021).

### 3. MATERIALS AND METHODS

#### 3.1. Study Area

The study was conducted from December 2022 to April 2023 in Jigjiga district of Fafan zone, Somali Regional State, Eastern Ethiopia, which is located at an elevation of 1803 meters above sea level, 630 kilometers southeast of Addis Abeba Figure 1. The average minimum and maximum temperatures in the region are around 20<sup>0</sup> and 35<sup>0</sup> degrees Celsius, respectively, and are high throughout the year. Geographically speaking, the region can be found at 9°21'N and 42°48'E, respectively (Tafesse, 2001). Rainfall is bimodal and averages 660 mm annually. Fafan Zone is known for its agro-pastoral production system, with livestock being the primary source of income for many residents. The camel population in the zone is about 290,649 (CSA, 2015).



**Figure 1:** Map of Study Area

### 3.2. Study Population

The population comprises lactating camels (*Camelus dromedaries*) residing in the Jigjiga district, managed under pastoral production systems. These animals migrate from areas with feed shortages to those with abundant resources, particularly during drought seasons.

### 3.3. Study Design

A cross-sectional study supported by a questionnaire survey was conducted from December 2022 to April 2023 to determine the prevalence of camel mastitis and identify the major bacterial pathogens contributing to mastitis and its associated risk factors in Jigjiga district, Fafan Zone, Eastern Ethiopia.

The questionnaire survey was aimed to assessing the management practices, potential risk factors for the occurrence of mastitis, and milk handling practices of the camel owners/herders. To ensure comprehensive data collection, potential risk factors such as age, body condition score; lactation stage, parity number, and use of an anti-suckling device (made by strips of cloth to apply on the udder/teat of a camel) were taken into account when questioning the camel owners or herders.

Using Faye *et al.* (2001) techniques of camel body condition scoring lactating camels were grouped into three categories: poor (score 1), medium (scores 2 and 3), and good (score 4). Based on rostral dentition (Bello *et al.*, 2013) and owner information, the lactating camels were categorized as young (<5 years) and adult ( $\geq 5$  years of age). The lactation stage was categorized into three categories: early (1-2 months), middle (3–9 months), and late (10–18 months) (Geresu *et al.*, 2021). Additionally, the parity number was grouped into three categories: category 1 (1<sup>st</sup> and 2<sup>nd</sup> parity), category 2 (3<sup>rd</sup> and 4<sup>th</sup> parity), and category 3 (greater than 4<sup>th</sup> parity) (Regassa *et al.*, 2013).

### 3.4. Sample Size Determination and Sampling Strategy

The sample size was calculated using the previous prevalence of 30.2% (Husein *et al.*, 2013), a 5% absolute precision level, and a 95% confidence interval (Thrusfield, 2008). Hence, using the standard formula indicated below (Thrusfield, 2008) and 30.2% as the expected prevalence (Husein *et al.*, 2013):

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

Where  $d$  represents the intended absolute precision level at a 95% confidence interval (0.05),  $P_{exp}$  is the expected prevalence (30.2%), and  $n$  is the necessary sample size. As a result, 324 lactating camels were selected for the study site.

The selection of peasant associations for this study was purposeful, taking into account factors such as camel milk marketing, accessibility of roads, and infrastructure. Out of a total of 30 peasant associations obtained from the Somali Region Agricultural and Rural Development Office, 15 were chosen for the study, with 10 from urban areas and 5 from rural areas. Among the selected peasant associations, households that owned lactating camels and voluntarily participated in the interview were purposefully included in the study. To ensure that the data collected is representative of the entire population of lactating camels in the area, from each selected household, a proportional number of lactating camels were selected using simple random sampling for the collection of milk samples and clinical examination of the udder.

The determination of the sample size for households in this study was based on the formula recommended by Arsham (2007) for survey studies. The formula is as follows:

$$N = \frac{0.25}{SE^2}$$

Where  $N$  represents the sample size and  $SE$  represents the proportional standard error and 0.25 is a constant that reflects a desired level of precision (95% confidence). In accordance with this formula, a total of 34 households with lactating camels were selected for an interview. The standard error was assumed to be 8.6% at a precision level of 5% and a confidence interval of 95%.

### **3.5. Clinical Examination of the Udder**

Clinical mastitis in camels is defined as an udder quarter with visible abnormal inflammatory changes in the mammary gland tissue such as redness, swelling, heat, and pain (Geresu *et al.*, 2021). The milk from the affected quarter is discoloured and clotted (El Tigani-Asil *et al.*, 2020).

Clinical mastitis was diagnosed through visualization and palpation of the camel udder. The examination process involved a comprehensive assessment of the udder's size, symmetry, and the presence of any lesions. Additionally, milk quality, colour changes, and the presence of any clots, flakes, blood or pus were carefully noted. Furthermore, blind

teats were also regarded as clinical mastitis because the blockage of the teat is the chronic stage of mastitis, which can be clinically identified (Radostits *et al.*, 2007; Abera *et al.*, 2010; Balemi *et al.*, 2021).

### 3.6. California Mastitis Test (CMT)

Subclinical mastitis is characterized by apparently normal milk and absence of clinical symptoms in the udder, increased in leukocyte number as evident by CMT and positive culture result (Seligsohn *et al.*, 2021).

The diagnosis of sub-clinical mastitis is a crucial aspect of camel health management. This involves determining the coagulation type and viscosity of the milk and CMT mixture, which reveal the presence and degree of infection. To carry out this process, Jilo *et al.* (2017) approach was utilized, which involves adding equal parts (5 ml) of milk and CMT reagent to each paddle well, arranged in quarters with a slight rotation movement of the paddle. The interpretation of the results is such that a CMT score of 0 is considered negative, while scores of trace, 1+, 2++, and 3+++ are considered positive Table 1. It is important to note that camels are considered positive for subclinical mastitis when at least one teat of an animal is positive for CMT (Ahmed *et al.*, 2019).

**Table 1:** Classic Grading and Interpretation of CMT Score

Score	Meaning	Visible reaction	SSC Cells/ml
-	No reaction	Mixture remains liquid	<200,000
T	Trace	A slight slime	150,000-500,000
+	Weak	A distinct slime	400,000-1.5*10 <sup>5</sup>
++	Distinct +ve	Mixture thickens with gel formation.	800,000-5*10 <sup>6</sup>
+++	Strong +ve	A distinct gel formations, which tends to adhere to the bottom of the paddle	>5*10 <sup>6</sup>

Source (Jilo *et al.*, 2017).

### 3.7. Milk Sample Collection

The sterile milk sampling protocol, as defined by Kirk (2000), was followed to collect milk samples. In order to stimulate milking, a camel calf was allowed to suckle, and samples were taken from all quarters to screen for subclinical mastitis. To ensure proper hygiene, the teat of the camels were carefully washed with clean water and soap, dried, and

disinfected with cotton swabs soaked in 70% alcohol. Approximately 10ml of milk was collected after discarding the first stream. The samples were immediately placed in an ice box (4-8<sup>0</sup>C) and transported to Jigjiga Regional Veterinary Diagnostic and Research Laboratory for processing and storage. CMT screening was done in the laboratory before storage and further processing.

### **3.8. Bacteriological Examinations**

All CMT-positive milk samples were subjected to bacteriological culturing according to Quinn *et al.* (2004). A loop full of each milk sample was streaked primarily on a plate of sheep blood agar and MacConkey agar and then incubated at 37°C for 24–48 hours. The plates were carefully examined for any bacterial growth, morphologic features of the bacteria on agar, pigmentation, or hemolytic characteristics of the colonies. Subcultures were then made on nutrient agar to obtain pure isolates for further bacteriological identification, following the standard procedures described by Quinn *et al.* (2004).

To identify the genera of bacterial species, Gram staining, catalase, oxidase, oxidation fermentation (OF), and motility tests were conducted, as recommended by Quinn *et al.* (2004). The *Enterobacteriaceae* family and *staphylococcal spp.* was identified using Triple Sugar Iron (TSI) and coagulase tests, respectively. *Staphylococcus spp.* was further identified by growth on Mannitol Salt Agar. *Streptococci* isolates were evaluated based on the Christie Atkins Munch Peterson (CAMP) reaction and hydrolysis of esculin. Additionally, Eosin-methylene blue (EMB) agar, Indol, Metyl-Red (MR), Voges-Proskauer (VP), citrate utilization, nitrate reduction test and urease tests were conducted to identify *Enterobacteriaceae*, following the methods outlined by Balemi *et al.* (2021).

### **3.9. Data Analysis**

The data collected from the questionnaire survey, clinical examination, CMT screen and laboratory investigations were meticulously recorded and coded using Microsoft Excel spread sheet software, provided by Microsoft Corporation. The R software version 4.1.1 was utilized to analyse the data. Descriptive statistics was used to summarize the data and the overall prevalence of mastitis was calculated by dividing the number of clinical and subclinical mastitis cases by the total number of animals examined.

To determine the association between prevalence and potential risk factors such as age, body condition score, lactation stage, parity, and use of anti-suckling devices, univariable logistic regression was employed. Furthermore, multivariate logistic regression analysis was utilized to calculate the odds ratio, which measures the degree of association between risk factors and camel mastitis, as well as the 95% confidence interval. The risks factors with an OR equal to 1 are considered unlikely to be associated with camel mastitis. If the OR is greater than 1, the risk factor is associated with increased odds of camel mastitis. Conversely, if the OR is less than 1, the risk factor is associated with decreased odds (protective) of camel mastitis.

### **3.10. Ethical Clearance**

Ethical clearance for the study was approved by and obtained from Addis Ababa University College of Veterinary Medicine and Agriculture animal research ethical review committee with an approved certificate reference number of VM/ERC/17/03/13/2021. The samples for this study were carefully collected without causing any harm to the animals, following all ethical procedures for sampling, enrollment, and sample collection. Before sample collection from the animals, the objectives of the study were discussed, and informed consent was sought from the owners.

## 4. RESULT

### 4.1. Animal Level Prevalence of Clinical and Subclinical Mastitis

Clinical examinations of the udder and the California Mastitis Test (CMT) were used to evaluate 324 lactating camels for the presence of camel mastitis. The overall prevalence of mastitis at the camel level was found to be 33.6% n= 109, (95%CI: 28.5%–39.1%). The prevalence of clinical mastitis was 6.5%, n=21, (95%CI: 4.0%–9.7%), and that of subclinical mastitis was 27.1%, n= 88, (95%CI: 22.4%–32.3%) at camel level.

### 4.2. Quarter-Level Prevalence of Mastitis

All quarters were examined for the existence of gross abnormalities, and it was observed that the quarter-level prevalence examined by CMT was 22.7%, n=290, (95%CI: 20.4%–25.1%) Table 2.

**Table 2:** Quarter Level Prevalence of Camel Mastitis

Quarter	NQE	NBQ (%)	CMTE	CMTP	Prevalence%(95% CI)
Left front	324	4(1.2)	320	68	21.3(16.9-26.1)
Right front	324	3(0.9)	321	68	21.2(16.8-26.1)
Left hind	324	6(1.6)	318	80	25.2(20.5-30.3)
Right hind	324	6(1.6)	318	76	23.9(19.3-29.0)
<b>Total</b>	<b>1296</b>	<b>19(1.5)</b>	<b>1277</b>	<b>290</b>	<b>22.7(20.4-25.1)</b>

NQE: Number of Quarter Examined, NBQ: Number of Blind quarter, CMTE: CMT Examined, CMTP: CMT positive

### 4.3. Risk Factors Associated with the Occurrence of Mastitis

Analysis of univariable logistic regression revealed that the occurrence of lactating camel mastitis was found to be significantly ( $p<0.05$ ) associated with risk factors, as shown in Table 3. Higher mastitis prevalence was found in adults as compared to the young age group (COR: 4.8; 95%CI: 2.85–8.18), with a significant difference of  $P<0.05$ . Similarly, a higher prevalence of mastitis was found in poor body condition score camels compared to good body condition camels (COR: 3.07; 95%CI: 1.70–5.55), with a significant difference of  $P<0.05$ .

**Table 3:** Uni-variable logistic regression

Category	Factors	NAE	NP%	Coef.(95%CI)	COR (95%CI)	P-value
Age	Young	148	24(16.2)	Reference	Reference	
	Adult	176	85(48.3)	1.57(1.05-2.10)	4.83(2.85-8.18)	0.000
BCS	Good	122	28(23.0)	Reference	Reference	
	Medium	112	38(33.9)	0.54(-0.03-1.12)	1.72(0.97-3.06)	0.064
	Poor	90	43(47.8)	1.12(0.53-1.71)	3.07(1.70-5.55)	0.000
Lactation	Early	116	58(50.0)	Reference	Reference	
	Mid	100	26 (26.0)	-1.20(-1.8-(-0.6))	0.30(0.17- 0.54)	0.000
	Late	108	25(23.1)	-1.05(-1.6-(-0.5))	0.35(0.20- 0.63)	0.000
Parity	1 <sup>st</sup> & 2 <sup>nd</sup>	75	11(14.7)	Reference	Reference	
	3 <sup>rd</sup> & 4 <sup>th</sup>	111	27(24.3)	0.63(-0.15-1.40)	1.87(0.86-4.05)	0.112
	>4 <sup>th</sup>	138	71(51.4)	1.82(1.10-2.54)	6.17(3.00-12.69)	0.000
ASD	Absent	239	51(21.3)	Reference	Reference	
	Present	85	58(68.2)	2.07(1.52-2.62)	7.92(4.56-13.75)	0.000
<b>Total</b>		<b>324</b>	<b>109</b>			

COR:Crude Odds Ratio, NAE: Number of Animal Examined, NP: Number of Positive

Multivariate logistic regression was used to assess the association of risk factors with the disease and calculate the adjusted odds ratio and 95% confidence interval. Among the predictive variables of camel mastitis age and parity numbers were highly correlated, consequently, parity number was dropped from the final model of multivariable logistic regression. Adult camels were nearly five times (95%CI = 2.81–9.90, P = 0.000) more likely to be infected than their young counterparts. The odds of mastitis occurrence were nearly 4 times (AOR: 3.40) (95%CI = 1.61–7.17, P = 0.001) and 4.20 (95%CI = 1.97-8.94, P = 0.001) for medium and poor body condition scores, respectively, as compared to a good body condition score. Mid lactation (AOR: 0.22, 95%CI = 0.10–0.45, P = 0.000) and late lactation (AOR: 0.23, 95%CI = 0.11–0.48, P = 0.000) were statistically found to be protective as compared to the early lactation stage. Likewise, utilization of an anti-suckling device exhibited a significant association (AOR: 10.08; 95%CI = 5.16-19.69, p = 0.000) with the prevalence of mastitis Table 4.

**Table 4:** Multivariate Logistic Regression

Category	Factors	NAE	NP%	Coef.(95%CI)	AOR (95%CI)	P-value
Age	Young	148	24(16.2)	Reference	Reference	
	Adult	176	85(48.3)	1.66(1.03-2.29)	5.27(2.81-9.90)	0.000
BCS	Good	122	28(23.0)	Reference	Reference	
	Medium	112	38(33.9)	1.22(0.48-1.97)	3.40(1.61-7.17)	0.001
	Poor	90	43(47.8)	1.43(0.68-2.19)	4.20(1.97-8.94)	0.000
Lactation	Early	116	58(50.0)	Reference	Reference	
	Mid	100	26 (26.0)	-1.53(-2.26-(-0.80))	0.22(0.10- 0.45)	0.000
	Late	108	25(23.1)	-1.47(-2.19-(-0.74))	0.23(0.11-0.48)	0.000
ASD	Absent	239	51(21.3)	Reference	Reference	
	Present	85	58(68.2)	2.31(1.64-2.98)	10.08(5.16-19.69)	0.000
<b>Total</b>		<b>324</b>	<b>109</b>			

AOR: Adjusted Odds Ratio, NAE: Number of Animal Examined, NP: Number of Positive

#### 4.4. Bacterial Isolation and Identification

Bacteriological examination was performed on a total of 109 selected (one teat from each positive camel) quarter milk samples from 290 CMT-positive quarters; 89% (97/109) of the samples produced mastitis pathogens, whereas 11% (12/109) of the samples showed no sign of growth. As shown in Table 5, *coagulase-negative Staphylococci* (26.8% of 26/97) were the dominant bacteria isolated, whereas *Streptococcus agalactiae* (5.1% of 5/97) was the least.

**Table 5:** Bacterial Isolates from Quarter Milk Samples of Lactating Camels

Bacterial isolates	Frequency	% of total isolates
<i>Coagulase-negative Staphylococci</i>	26	26.8
<i>Streptococcus spp. other than S. agalactiae</i>	19	19.6
<i>Staphylococcus aureus</i>	13	13.4
<i>Escherichia coli</i>	12	12.4
<i>Corynebacteria spp.</i>	8	8.2
<i>Bacillus spp.</i>	7	7.2
<i>Micrococcus spp.</i>	7	7.2
<i>Streptococcus agalactiae</i>	5	5.2
<b>Total</b>	<b>97</b>	<b>100</b>

#### 4.5. Household's Questionnaire Survey Result

In the survey of 34 camel owning households, 88.2% (30/34) responded that they were aware of clinical mastitis and that it was a significant problem in the study area Table 6.

**Table 6:** Questionnaire Survey Result in Jigjiga District

<b>Milking procedure</b>	<b>Result of respondents (%)</b>	
	Yes	No
Wash udder & teats before milking	0(0.00)	34(100)
Let the calf suckle before milking	34(100)	0(0.00)
Wash milk utensils before milking	34(100)	0(0.00)
Wash hands before and at intervals of milking	2(5.9)	32(94.1)
Mastitic camel milk last	10(29.4)	24(70.6)
Awareness on clinical mastitis	30(88.2)	4(11.8)
Awareness on subclinical mastitis	0(0.00)	34(100)

## 5. DISCUSSIONS

Camel mastitis is a disease that affects the mammary gland of camels, which can lead to a decrease in milk production and quality, which causes economic losses largely due to subclinical mastitis (El Tigani-Asil *et al.*, 2020). It is an important constraint to milk production in arid and semiarid parts of Ethiopia (Geresu *et al.*, 2021). Consequently, in the current study, the prevalence of mastitis in lactating camels was 33.6% (95%CI: 28.5%–39.1%) overall. The outcome was consistent with reports by Wubishet *et al.* (2016) that claimed 34.7% in the Borena zone of Oromia Regional State and 33.3% in and around Hargesa, Somaliland, by Mogeh *et al.* (2019). The current prevalence finding is higher than the reports of 22.4% in the Gomole District of Borena Zone, Southern Ethiopia, by Geresu *et al.* (2021), 29% in Jijiga Zone, Somali Regional State, Ethiopia, by Abera *et al.* (2010), and 18.5% in Abu Dhabi, United Arab Emirates, by Al-Juboori *et al.* (2013). However, it was lower than the prevalence report of 76% in selected pastoral areas in eastern Ethiopia by Seifu and Tafesse (2010) and the 59.8% reported by Bekele and Molla (2001) in the Afar Region, north-eastern Ethiopia. Despite the similarity in pastoral production systems in these areas, there are differences in how camel herders manage and control mastitis. Access to veterinary care and environmental conditions can also impact mastitis rates in different regions (Geresu *et al.*, 2021). Unhygienic milking procedures and generally poor management practices can contribute to a higher prevalence of mastitis in camel herds (Alebie *et al.*, 2021). The higher herd size in each household in the study area may contribute a higher report of prevalence of camel mastitis. The climate in arid and semiarid regions can affect the prevalence of camel mastitis in several ways, as camels live in these areas which are not suitable for crop production and where other livestock species hardly thrive (Seifu and Tafesse, 2010).

The current study has revealed a clinical mastitis prevalence of 6.5% (95%CI: 4.0%–9.7%), which is comparable to the estimate of 6.3% reported by Mehamud *et al.* (2017) in Gursum District, Eastern Hararghe, Ethiopia, and nearly matches the 8.3% reported in Jijiga, Eastern Ethiopia, by Abera *et al.* (2010). However, it was higher than the prevalence reports of 2.1% in the Borena lowland, southwest of Ethiopia, by Woubit *et al.* (2001) and 4.9% in Jijiga town, east of Ethiopia, by Husein *et al.* (2013). Conversely, it is lower than the 12.5% reported by Bekele and Molla (2001) in the Afar region and the 19.5% in Eastern Sudan by Osman (2008). The variation in clinical mastitis prevalence across

different regions may be attributed to several factors, including management practices, environmental conditions, and access to veterinary services (Jilo *et al.*, 2017). Inadequate veterinary service and lack of awareness on the treatment of camel mastitis may contribute the higher record of clinical mastitis in the study area.

In the present study, the prevalence of sub-clinical mastitis in camels, as determined by the California Mastitis Test (CMT), was found to be 27.1% (95%CI: 22.4%–32.3%). This figure is comparable to the findings of Alebie *et al.* (2021) in the Afar Regional State of northeastern Ethiopia, where the prevalence was reported to be 25%, and to the results of Husein *et al.* (2013) in Jijiga Town, eastern Ethiopia, where the prevalence was 25.3%. However, the prevalence in this study was relatively higher than the reports of Geresu *et al.* (2021) in the Gomole District of Borena Zone, southern Ethiopia (18.1%), Almaw and Molla (2000) in eastern Ethiopia (24.1%), and Abera *et al.* (2010) in Jijiga, eastern Ethiopia (20.7%). Conversely, these findings were lower than the reports of Regassa *et al.* (2013) in the Yabello district of Borena Zone (39.4%) and Bekele and Molla (2001) in the Afar Region (47.3%). No awareness on the subclinical mastitis by camel owners in the study area increased the prevalence of camel clinical mastitis.

The present study considered the quarter-level prevalence of mastitis and revealed an overall prevalence of 23.8%. Among the affected quarters, 1.5% teats were blind, and 22.7% were CMT-positive. This result was consistent with the report's overall quarter-level prevalence of 24.0%, from which 2.2% blind teats and 22.3% were CMT positive in Borana Zone, Oromia Regional State, Ethiopia, by Regassa *et al.* (2013), and 25.3%, from which 1.7% blind teats and 24.7% were CMT positive in Gursum District, Eastern Hararghe, Ethiopia, by Mehamud *et al.* (2017). However, it was higher than the reported overall quarter-level prevalence of 16.6%, from which 2.9% blind teats and 14.1% CMT positive quarters were reported in Gomole District of Borena Zone, Southern Ethiopia, by Geresu *et al.* (2021), and 17.9% quarter-level prevalence with a high proportion report of 33.8% blind teats in Jijiga, Eastern Ethiopia, by Abera *et al.* (2010). Whereas, the result was lower than the report of an overall quarter-level prevalence of 25.8% with 1.8% blind teats and 24.8% CMT positives in Jijiga town in eastern Ethiopia by Husein *et al.* (2013) and 45.3% with 4.5% blind teats and 30.4% CMT positives by Ahmad *et al.* (2011). The differences in the reported prevalence of mastitis may be attributed to variations in management practices, herd health status, and diagnostic methods used by different studies (Seligsohn *et al.*, 2021).

The current study revealed that the hind quarters, left hind (25.2%) and right hind (23.9%), were more inflamed than the front quarters, the right front (21.2%) and left front (21.3%), which agreed with the report of the right hind (4.3%) and left hind (4.3%) than the right (4.1%) and left front (3.9%), irrespective of the prevalence by Geresu *et al.* (2021). However, this result did not agree with the report of the right quarters: the right front (15.6%) and right hind were more exposed than the left quarters, the left front (5.2%) and left hind (4.6%) by Mogeh *et al.* (2019). The higher risk of infections in the hind quarters compared to the front ones could be due to unfavourable hygienic conditions and greater exposure to dung and urine. In addition, because of the shorter duration of the hind teats and the correspondingly shorter teat canal, the defence potential in the hind quarter could be decreased (Wanjohi *et al.*, 2013).

The results of the current investigation showed that the prevalence of camel mastitis was significantly associated ( $p < 0.05$ ) with the hypothesized risk factors of age, body condition score, lactation stage, and usage of ant-suckling devices to varying degrees. The prevalence of mastitis was associated with age; adult ( $\geq 5$  years) camels were nearly 5 times (AOR = 5.27; 95%CI= 2.81-9.90  $P=0.000$ ) more likely to be infected than their young counterpart ( $< 5$  years), which is consistent with other studies (Zeryehun *et al.*, 2017; Geresu *et al.*, 2021). To combat bacterial pathogens, the udder's physiological defense mechanisms become less effective with age, and the sphincter becomes more relaxed, which allows pathogenic organisms to enter the glandular tissue and cause inflammation of the mammary glands (Radostits *et al.*, 2007). As camels age, their udder tissue becomes more susceptible to damage, making them more vulnerable to mastitis. Hormonal changes such as imbalances of endocrine, cytokines, and antioxidants also impact the immune system's response to infection, making older camels more vulnerable (Gramay and Ftiwi, 2018).

This study found a significant association between body condition scores ( $p = < 0.05$ ). The odds of mastitis occurrence were nearly 4 times (AOR: 3.40) (95%CI = 1.61–7.17,  $P = 0.001$ ) and 4.20 (95%CI = 1.97-8.94,  $P = 0.001$ ) for medium and poor body condition scores, respectively, as compared to a good body condition score. This was in line with the findings of Zeryehun *et al.* (2017), who claimed a significant association between poor body condition score and the occurrence of mastitis in dromedary camels in the pastoral areas of the Borena lowland in southern Ethiopia and in the Cholistan desert in Pakistan by Ali *et al.* (2019). However, it is worth noting that Geresu *et al.* (2021) did not observe any

cases of mastitis in camels with poor (thin) body condition scores, which contradicts the aforementioned findings. Under feeding and poor body conditions provide favourable environment to bacterial pathogenesis development (Mbuk *et al.*, 2016).

The current study revealed that the lactation stage was significantly associated with the occurrence of camel mastitis ( $p < 0.05$ ). Mid lactation (AOR: 0.22, 95%CI: 0.10–0.45,  $P = 0.000$ ) and late lactation (AOR: 0.23, 95%CI: 0.11–0.48,  $P = 0.000$ ) were statistically found to be protective as compared to the early lactation stage. This report agreed with the reports elsewhere (Ahmad *et al.*, 2011; Zeryehun *et al.*, 2017; Saidi *et al.*, 2021). A higher prevalence during the first few weeks of lactation could be attributed to several variables, including high milk production, settings that are more conducive to infection occurrences, and a lack of immune response in reaction to calving stress. The majority of new infections happen in the first two months of lactation and in the early dry phase, particularly when environmental pathogens are involved (Radostits *et al.*, 2007).

Moreover, the utilization of an anti-suckling device exhibited a significant association ( $p = 0.000$ ; AOR = 10.08; 95%CI = 5.16–19.69) with the prevalence of mastitis in this study, which was consistent with the findings of Ahmed *et al.* (2019). This device is commonly employed in pastoral areas, where fibers from plants or strips of cloth are tied to the teat to prevent the calf from sucking the camel. However, this practice may cause damage to the teat, leading to an increased risk of mastitis (Abera *et al.*, 2010). Furthermore, the use of cloth strips tied to the animals' udders at the study site may contribute to the higher prevalence of mastitis. The cloth retains moisture, which may create a favourable environment for bacterial growth and multiplication.

Based on the findings of the current study's bacteriological isolations, *coagulase-negative staphylococci* and *Streptococcus spp.*, excluding *S. agalactiae*, were identified as the primary culprits, with isolation percentages of 26.8% and 19.6%, respectively. These organisms have been previously recognized as significant mastitis-causing pathogens in lactating camels by other researchers (Al-Rammahi *et al.*, 2018). In contrast, *Streptococcus agalactiae* had the lowest percentage of isolations (5.2%), which is consistent with a study conducted by Husein *et al.* (2013) in Jigjiga town, Eastern Ethiopia. The rise in the number of isolates of major mastitis-causing pathogens can be attributed to several factors. One of the primary reasons is the inadequate supply and infrequent use of antimicrobials.

Additionally, the inaccessibility of veterinary services for camel owners, particularly in rural and peri-urban areas, is a significant contributing factor (Mehamud *et al.*, 2017).

The occurrence of *Staphylococcus aureus* in this study was found to be 13.4%, which is comparable to the findings of Geresu *et al.* (2021) in the Gomole District of Borena Zone, Southern Ethiopia, where the prevalence was reported to be 11.92%. However, it was significantly higher than the findings of Almaw and Molla (2000), who reported a prevalence of 0.6%, and lower than the reports of Woubit *et al.* (2001) and Mengistu *et al.* (2010), who reported a prevalence of 16% and 21.03%, respectively. While it has been suggested that *Staphylococci spp.* are considered minor pathogens isolated from subclinical mastitis cases that do not have a significant impact on milk yield, CMT, or clinical symptoms (Eberlein, 2007), there are several factors that can influence the prevalence of *Staphylococcus aureus*. These factors include differences in study populations, sample size, sample collection methods, laboratory methods for identifying the bacteria, and hygiene practices. Furthermore, changes over time, such as the use of antibiotics or modifications in animal husbandry practices, can also impact the prevalence (Jilo *et al.*, 2017).

The current data indicated that 12.4% of *Escherichia coli* isolates were present; this finding was more comparable to the report of 10.5% by Geresu *et al.* (2021). However, it was greater than the report of 6.3% by Husein *et al.* (2013) and lower than the reports of 18% by Kalla *et al.* (2008) and 18.18% by Amel (2003). It is important to note that the occurrence of *E. coli* can vary significantly depending on hygiene conditions, as coliforms can be an indicator of insufficient hygienic conditions and a minor degree of contamination (Eberlein, 2007). Additionally, differences in sample size, location, and methods used for detection can cause variations in the prevalence of *E. coli* over time (Rahmeh *et al.*, 2022).

The current study revealed that 8.2% of *Corynebacterium spp.* were identified, which is comparable to the findings of Geresu *et al.* (2021) and Husein *et al.* (2013), who reported 9.2% and 9.0%, respectively. However, the proportion of *Corynebacterium spp.* discovered in this study was lower than that reported by Al-juboori *et al.* (2013) (10%) and Sanna (2005) (30.7%). It is important to note that various factors, such as sample size and composition, geography, environmental factors, laboratory procedures, and the immunological status of the host population, can influence the outcomes of different research studies (Rahmeh *et al.*, 2022).

The current study revealed a 7.2% isolation of *Micrococcus spp.*, which is consistent with the findings of Geresu *et al.* (2021) and Alebie *et al.* (2021), who reported a proportion of 7.3% and 9.2%, respectively. However, this proportion was higher than the 5.7% reported by Saleh and Faye (2011) in the Dubti district of Ethiopia's Afar Regional State. The lack of proper milking hygiene at the research site and not washing the hands, teats, and udders of lactating camels before milking may have contributed to the difference in bacterial isolates.

The prevalence of *Bacillus spp.* in various districts of Ethiopia has been the subject of several studies. Mehamud *et al.* (2017) reported 6.6% prevalence in the Gursum district of Eastern Hararghe, which is comparable to the 7.2% prevalence found in the current study. However, Alebie *et al.* (2021) reported a much higher prevalence of 19.57% in the Dubti district of Afar Regional State, while Mengistu *et al.* (2010) reported a lower prevalence of 4.3% in Gewane district, also in Afar Regional State. *Bacillus spp.* is known to be widely distributed in nature, with the majority of species found in soil, water, dust, air, faeces, and vegetation. As such, it should be considered a possible cause of camel intra-mammary infection (Barrett *et al.*, 2005).

During the course of this research, 34 households that owned camels were interviewed. The results showed that 88.2% of the respondents believed they had knowledge of clinical mastitis, which they identified as the predominant problem in the research area. Depending on the symptoms it showed the disease was known by various local names, such as "Gofla" (reduction in milk output and swelling of the udder), "Arar" (abnormal milk content), and "Jid" (blind teats). However, it was concerning to note that none of the respondents were familiar with subclinical mastitis, which is consistent with the findings of an earlier study conducted in Jijiga, Eastern Ethiopia, by Husein *et al.* (2013). This highlights the need for further education and awareness campaigns to address this issue. On a positive note, almost all respondents practiced letting the calf suckle before milking, and washing and smoking milking instruments before milking. However, it was surprising to find that only 5.9% of the respondents exercised hand washing before and after milking. This finding underscores the importance of promoting good hygiene practices to prevent the spread of disease. These findings are in line with the establishment of Geresu *et al.* (2021) in Gomole District, Borena Zone of southern Ethiopia.

## 6. CONCLUSION AND RECOMMENDATIONS

Camel mastitis is a serious health concern that affects lactating camels, and it is a prevalent disease in camels in Jigjiga district, both at the animal and quarter levels. The high prevalence of subclinical mastitis in the study area indicates significant economic losses due to its detrimental impact on productivity. Age, body condition score, lactation stage, and use of anti-suckling devices were the risk factors for the occurrence of camel mastitis. Poor milking hygiene practices, such as inadequate hand washing before and at the interval of milking, insufficient udder cleaning before milking, and a lack of practices to milk mastitic camels last, are major contributors to the occurrence of camel mastitis. The study identified both gram-positive and gram-negative bacteria as the primary culprits. Gram-positive bacteria, such as *Staphylococci spp.*, *Streptococcus spp.*, *Corynebacterium spp.*, *Bacillus spp.*, and *Micrococcus spp.*, were isolated, as well as gram-negative bacteria like *Escherichia coli*. Based on the above conclusions, the following recommendations are forwarded:

- Promote hand washing before and after milking: Farmers should be encouraged to wash their hands thoroughly with soap and water before and after milking to prevent the spread of bacteria.
- Ensure adequate udder cleaning before milking. Proper udder cleaning is essential to prevent the transfer of bacteria from the environment to the udder. Farmers should be trained on how to clean the udder properly before milking.
- Awareness should be created among farmers about the practice of mastitic camels to milk last. Mastitic camels should always be milked last to prevent the spread of infection to healthy camels.
- The use of anti-sucking devices should also be avoided to reduce the risk of infection.
- Furthermore, regular monitoring and testing of camels for mastitis should be conducted to identify and treat infections promptly. This will help prevent the spread of the disease and minimize economic losses.

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## 8. APPENDIXES

### Annex 1: Camel Age Estimation by Dentition

Age	Category	Features
At birth		No teeth
At 9 month		All the deciduate teeth have erupted
At 4 year	Young	All the deciduate incisors and canine have worn down
At 7 year	Adult	All the permanent incisors and canine teeth have erupted.
At 12 year		All the permanent incisors are in wear,
At 15 year		All the permanent incisors and canine teeth have worn down
At 20 year		All the permanent teeth are down and clearly separated from each other

### Annex 2: Body Condition Scoring Method

		Features of category with its anatomical parts		
Condition	Category	Recto-genital zone	Ischial and coxal tuberosity, shoulder, scapula, spinus and transverse vertebrae	Ribs
Score 1	Poor	Very deep	Very prominent	Individually visible
Score 2	Medium	Deep	Prominent	Slightly visible
Score 3	Medium	Slightly deep	Slightly prominent	Not very visible
Score 4	Good	Full of fat	Not visible	Not visible

### Annex 3: Cross-Sectional Questionnaires Survey

#### A. Household information (one form to be completed for each household)

1. Interviewer ..... date .....
2. Survey (household)..... division .....
3. Location..... sub location.....
4. Owners name.....sex..... age.....
5. Interviewee.....
6. Camel herd composition
  - a. Number of lactating camels.....
  - b. Number of dry camels.....
  - c. Breeding bulls.....
  - d. Immature male.....
  - e. Immature female.....
7. Camel milk production per day....
8. Frequency of milking per day.....

9. Let the calf suckle before milking yes.....no.....
10. Other livestock owned by the family
  - a. Cattle.....
  - b. Goat .....
  - c. Sheep.....
  - d. Donkey.....
  - e. Other.....
11. For how long have you been keeping camels
  - a. <1 year b. 1-5 year c. >5 year
12. Have you heard of mastitis in camel? Yes.....no.....
13. What is its local name.....
14. Have you ever had cases of mastitis? Yes.....no.....
15. If yes what sign do you see to know it is mastitis?
  - a. Swollen and painful udder/quarter
  - b. Bloody milk
  - c. Reduced milk
  - d. Other signs.....
16. Was any treatment given? Ye.....no.....
17. Who administered the treatment?
  - a. Veterinary officer.....
  - b. Animal health assistant.....
  - c. Community animal health worker.....
  - d. My self.....
  - e. Others .....
18. Did the camel recover?
19. Who milks the camel?
  - a. Children .....
  - b. Wife.....
  - c. Husband.....
  - d. Other.....
20. If you have more than one milking camel, when a camel has mastitis, do you milk the camel last?  
Yes..... No.....
21. Are the camels milking proper method? (Observe).....
22. Do you wash the camel udder before milking? Yes.....no.....
23. How often do you remove the manure from its house where the milking camel spent at night?
  - a. Less than 1 month
  - b. 1-3 month
  - c. 3-6 month
  - d. Greater than 6 months

**B. Individual camel information (one form to be completed for each camel)**

1. Camel id .....

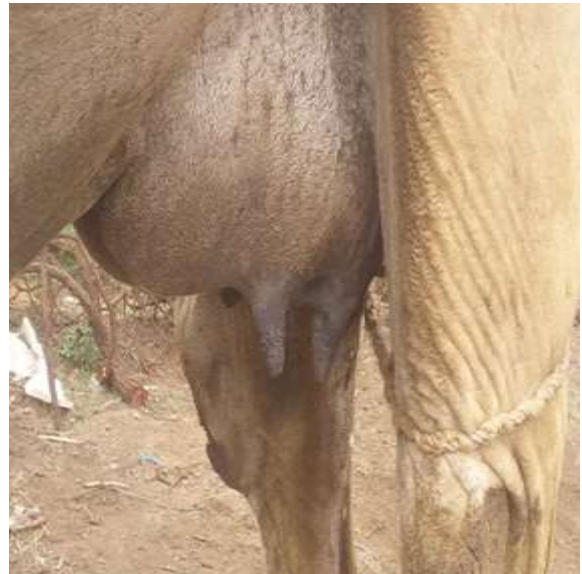
2. Lactation stage
  - a. Early (1-2 months).....
  - b. Middle (3-9 months).....
  - c. Late (10-18 months).....
3. Parity number
  - a. Category 1 (1<sup>st</sup> and 2<sup>nd</sup> parity).....
  - b. Category 2 (3<sup>rd</sup> and 4<sup>th</sup> parity).....
  - c. Category 3 (more than 4<sup>th</sup> parity).....
4. Age
  - a. Young (<5 years).....
  - b. Adult (≥5 years).....
5. Body condition
  - a. Poor (score 1).....
  - b. Medium (scores 2 and 3).....
  - c. Good (score 4).....
6. Use of ant-suckling device yes.....no.....
7. Current milk production per day.....
8. The current number of functional teats (observe).....
9. Number of blind teats.....
10. Has the camel ever had mastitis during the current lactation? Yes.....no.....
11. Any abnormalities in the teats? (Observe).....
12. If yes describe the lesion.....
13. Any abnormalities in the udder? (Observe).....
14. If yes describe the lesion.....
15. Carryout CMT and the result
  - a. Right forequarter .....
  - b. Left forequarter .....
  - c. Right hindquarter .....
  - d. Left hindquarter .....
16. Any other additional.....

**Annex 4: Laboratory Result of Bacteria**

Laboratory Result on Bacterial Culture and Identification Format

Sample no	RFQ	LFQ	RHQ	LHQ	Clinical	subclinical	Organism	Remark

**Annex 5: Sample Collection and Processing Pictures**



Let the Calf to Suckle before Milking



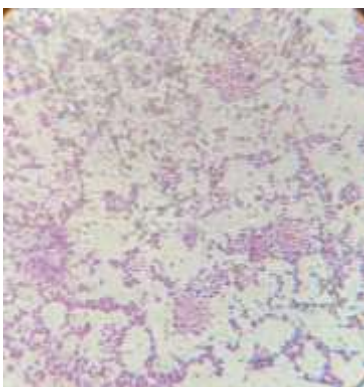
Anti-suckling device applied on the animal udder

**Annex 6: Photographs Indicating CMT Screening Pictures**

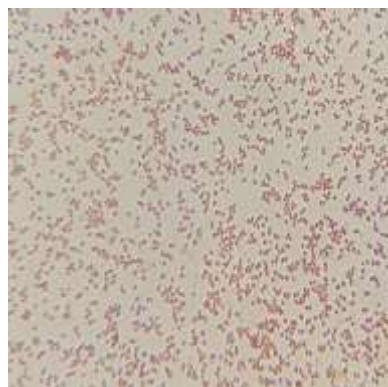


A distinct gel formation to CMT in left and no reaction in left

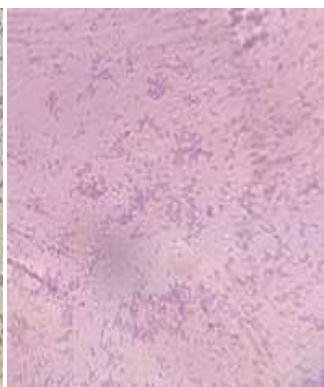
**Annex 7: Bacterial Culture and Media Pictures**



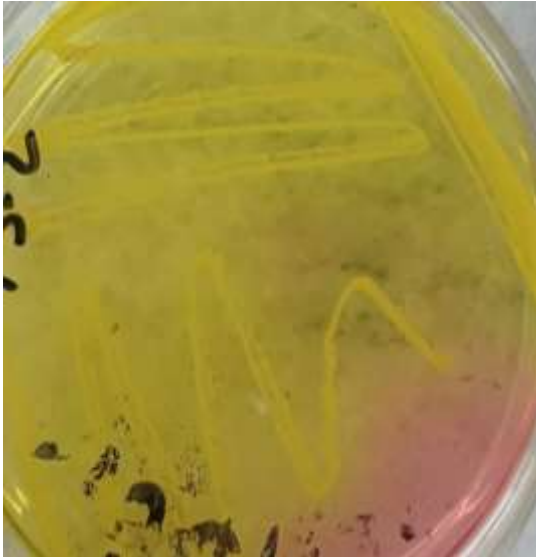
Gram Positive Bacteria



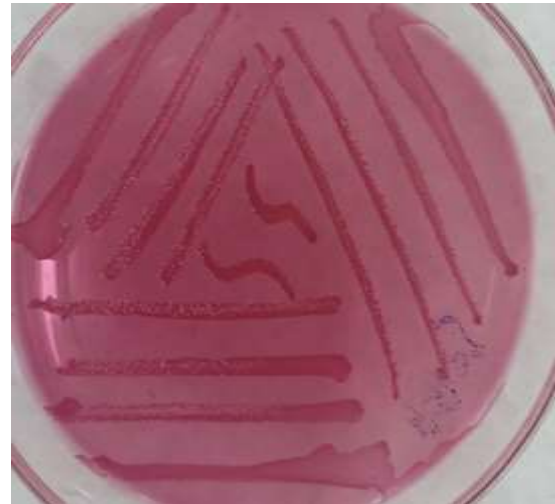
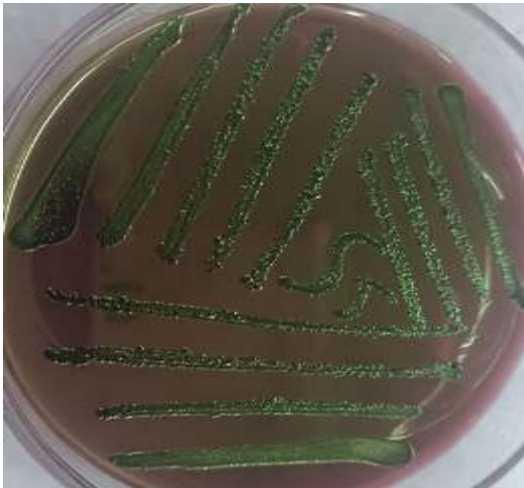
Gram Negative Bacteria



Cornebacterium spp.

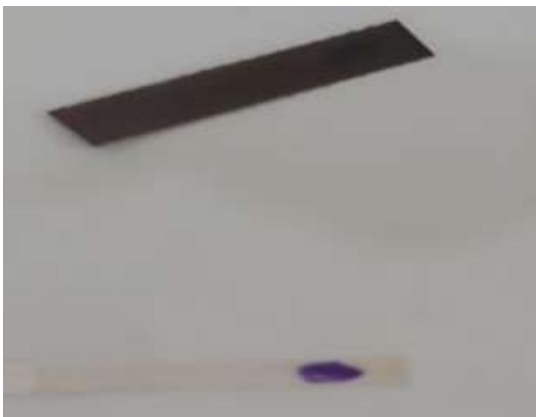


*S. aureus* on Manitol salt agar left and on nutrient agar on the right



*E. coli* on Eosin-methylene blue (EMB) agar on left and MacConcay agar on the right

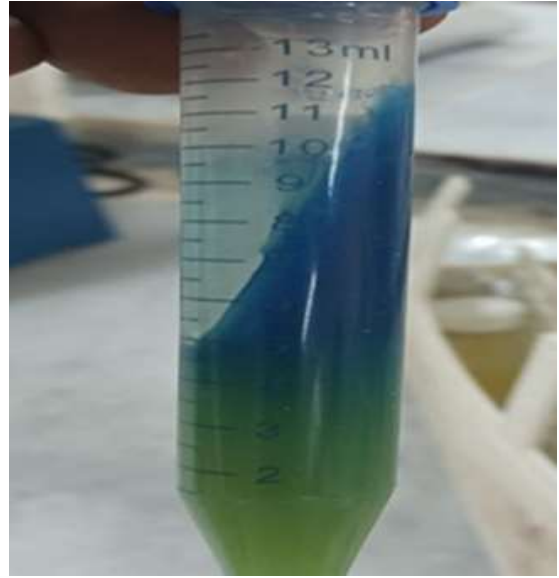
### **Annex 8: Biochemical Tests Pictures**



Oxidase Test



Catalase Test



Biochemical Tests used during the isolation of Bacteria

**Annex 9: Ethical Clearance**

<p>አዲስ አበባ ዩኒቨርሲቲ የእንስሳት ሕክምናና ግብርና ኮሌጅ ቢሾፍቱ</p>		<p>ADDIS ABABA UNIVERSITY College of Veterinary Medicine and Agriculture Bishoftu</p>
<p>Animal Research Ethical Review Committee</p>		
<p><i>Ethical clearance certificate</i></p>		
<p>Certificate Ref. No: VM/ERC/27/04/15/2023</p>		
<p>Name and affiliation of applicant: <b>Mulualem Kidane (DVM, MSc student)</b> Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University</p>		
<p>Title of the project: <i>Camel mastitis: prevalence, risk factors and isolation of major bacterial pathogens in Jigjiga District of Fafan Zone, Eastern Ethiopia.</i></p>		
<p>Date of application: Nature of the project: Target animal species: Number of animals involved: Study area:</p>	<p><b>December, 2022</b> <b>Field investigation</b> <b>Camel</b> <b>320</b> <b>Jigjiga, Ethiopia</b></p>	
<p>Minutes No. and date of review: VM/ERC/04/15/023, 15/02/2023</p>		
<p>The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Mulualem Kidane.</p>		
<p><u>Professor Getachew Terefe</u> Chairman</p>	<p>(DVM, PhD)</p>	 Signature
		
<p>መልሱን በሚጽፉልን ጊዜ እኩምን የኛን ደብዳቤ ቁጥር ይጥቀሱልን Please quote Our Ref. No. When replying</p>		
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<p>ቢሾፍቱ   ኢትዮጵያ Bishoftu, Ethiopia</p>		

## Annex 10: Plagiarism Report

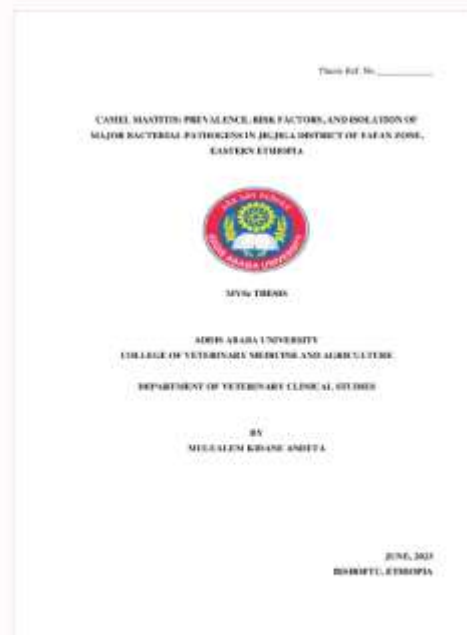


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