DETERMINATION OF RISK FACTORS ASSOCIATED WITH MASTITIS AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF BACTERIA ISOLATED FROM MASTITIC MILK IN DAIRY CAMELS IN BENAADIR REGION IN SOMALIA

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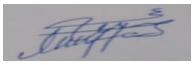
DECLARATION

I declare that this is my original work and has not been presented before in this university or any other university for the award of this or any other degree.

Signature……  ……Date……26/09/2022…..

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DEDICATION

This dissertation is dedicated to my parents

MOHAMED SAID YUSUF

And my Dear mom

WARIS MOHAMED SAID

they supported and encouraged me in pursuing academics. This is also a dedication to my young son.

ABDULLAHI MUSTAFE MOHAMED

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ABBREVIATIONS

FAO	Food Agricultural Organization
ICPALD	IGAD Centre For Pastoral Area And Livestock Development
CM	Clinical Mastitis
SCM	Sub Clinical Mastitis
CMT	California Mastitis Test
SCC	Somatic Cell Count
DNA	Deoxyribonucleic Acid
DMSCC	Direct Microscopic Somatic Cell Count
VPMP	Department of Veterinary Pathology, Microbiology and Parasitology

ABSTRACT

Camels can withstand arid and semi-arid harsh climatic conditions where crop production and other livestock species are limited. There are two camel species- *Camelus bacterianus* (Bactrian) and *Camelus dromedarious* (Arabian) - which have two and one humps, respectively. These species can produce milk even under extreme conditions. However, mastitis, an inflammation of the udders, has been globally reported among the key zoonotic diseases that threaten the dairy industry. This study was designed to establish the prevalence of mastitis in dromedary in Benaadir Region, Somalia, factors associated with its occurrence and the antimicrobial susceptibility profiles of mastitic milk isolates.

The study was cross-sectional; including usage of semi-structured questionnaires and bacterial isolation from milk samples. The questionnaires were administered to 96 camel keepers in Huriwa, Dharkenley, and Yaqshid regions in Benaadir district- Somalia, while a total of 290 she-camels had their milk sampled and processed for bacterial isolation. The milk samples were collected directly from cleaned udders early in the morning. After discarding the first four streams of milk, the California Mastitis Test (CMT) was carried out, and then approximately 10 ml of milk from each half was collected into labelled sterile tubes and transported in frozen conditions to the Department of veterinary pathology, microbiology and parasitology (VPMP), University of Nairobi, for bacterial isolation and identification.

Using CMT, the overall prevalence of subclinical mastitis was 29.0% (83/286). The prevalences of subclinical mastitis were 28.1% (27/96), 29.5% (28/96) and 30.2% (29/96) in Huriwa, Dharkenly and Yaqshid districts, respectively. From the milk samples, the highest bacterial isolates (50.0%) were from Huriwa district, whereas Dharkenly and Yaqshid districts had 26.3%, and 27.5%, respectively. The bacterial isolates included *Staphylococcus aureus, Streptococcus spp*.,

and *Escherichia coli*. Notably, these bacterial isolates were not only resistant to most of the tested antibiotics but also had multi-drug-resistant strains. The three were resistant to Penicillin at 34.62%, 100% and 100%, respectively. *Staphylococcus aureus s*, which exhibited resistance to most of the tested antibiotics, showed resistance to Ampicillin, Erythromycin and Streptomycin at 42.31%, 7.69% and 42.31%, respectively. Based on the odds ratio, the questionnaire data showed that respondents practising semi-intensive farming were 27 times more likely to experience mastitis among camels [Exp (B)= 27.28; 95% CI: 1.360, 547.08; p=0.031]. Among the farming systems, the majority 65% of respondents reported mastitis in camels and practised semi-intensive farming systems whereas both intensive and extensive farming systems had mastitis at less than 20% each.

The study has shown that clinical and sub-clinical camel mastitis cases are prevalent in Benaadir and that the most common causative agents are *Streptococcus spp, Staphylococcus spp,* and *Escherichia coli spp*. Since a semi-intensive farming system was established as a factor associated with the occurrence of mastitis in the region, efforts need to be made to educate the camel keepers not only on hygiene and good milking practices but also on good farming practices, to minimize the potential for getting mastitis among camels. The data is expected to help the authorities in the Benadir region in coming-up with respective control measures for the region.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Camels (*Camelus* species) have the potential to withstand harsh environmental conditions that are predominantly in arid and semi-arid areas where crop production and survival of other livestock species are limited (Mogeh, 2019). According to Ismail and Dickson (2010), there are two camel species- *Camelus bacterianus* (Bactrian) and *Camelus dromedarious* (Arabian) – characterised by two humps and one hump, respectively. Approximately 70% of Somalia's economy is from livestock and livestock products (Nur, 2005). In Somalia, the camel population is estimated to be 5-7 million whereby half of these are in Southern and Central Somalia, where inhabitants are pastoralists with large herds of camels purposely-kept for milk production, transport and drudgery.

Mastitis, an inflammation of the udders, has been globally reported among the key zoonotic diseases that threaten the dairy industry (Hussain *et al.,* 2017). In Somalia, where most animals can hardly survive, the camel serves as an ideal and reliable source of meat and milk (Dubad, 2019). Camel milk is not only sold in Muqdisho; there is also high demand in other places. Owing to the rise in demand for camel milk in Somalia, there are several campaigns on its consumption to widen its market. In line with camel milk consumption, Makau (2017) showed that the majority of people, especially in South-Eastern Somalia, integrated camel milk into their diet.

1.2 Statement of the problem

In Somalia, the majority of the population consumes camel milk; this follows its potential of milk production of 0.958 million metric tonnes behind Kenya as the leading global producer with 1.165 MMT. Its heavy consumption is attributed to its natural therapeutic and immunity-boosting properties contributed by a high concentration of lactoferrin, lactoglobulins and lysozyme. Somalia

is an arid and semi-arid region heavily dominated by pastoralism, such as camel rearing - their milk is available for extended periods compared to cattle (Wayua *et al.,* 2012). Following the increase in its demand, potential sources of contamination and diseases such as mastitis have been reported to significantly cause losses in the camel milk value chain (Aqib *et al.,* 2022). For example, Kenya, the leading global producer, has reported about 50% postharvest losses in all the camel milk produced (Akweya *et al.,* 2019; Odongo *et al.,* 2016). Among these postharvest losses were poor hygiene and socio-demographic factors. Hadef *et al.* (2022) identified unhygienic udder conditions as the major risk factor for the incidence of subclinical mastitis. There is little information on mastitis in camels in Somalia (Arush *et al.,* 1948; Abdurrahman *et al.,* 1992), however, there are several cases of mastitis reported in East Africa, the Middle East and Egypt (Moustafa *et al.,* 1987; Karmy, 1990). None of these studies covered factors associated with the increased prevalence and occurrence of mastitis. Similarly, little is documented on pathogens involved in mastitis occurrence in camels using culture-independent approaches. Notably, the available information on clinical and subclinical mastitis-causing microorganisms was exclusively reported based on conventional bacteriological (culture-dependent) methods, mainly from Ethiopia (Tigani-Asil *et al.,* 2020; Abera *et al.,* 2010; Alebie *et al.,* 2021; Seligsohn *et al.,* 2020; Geresu *et al.,* 2021). Studies have shown *Staphylococcus aureus* isolated from camel milk as multidrug-resistant (Ali *et al.,* 2018; Kuroda *et al.,* 2001). Given the many causal agents of camel mastitis, *Staphylococcus aureus* being included, several drugs in the form of vancomycin have been used but have shown no effectiveness.

1.3 Justification of the Study

Camel milk immensely contributes to the diet of a majority of pastoralists in the South-Eastern province of Somalia owing to its beneficial nutrients. Its demand has resulted in an upsurge in its

production which had necessitated proactiveness in ensuring safety, given that camel milk is highly susceptible to bacterial contamination. Therefore, advancing the knowledge on factors associated with the occurrence of camel mastitis offers an avenue for mitigating the prevalence in a socioeconomic and cultural dimension among the majority of the pastoralists. Additionally, the generated knowledge on bacterial causal agents of mastitis and their antimicrobial susceptibility profiles will aid in the management approaches.

1.4 Objectives

1.4.1 Overall objective

To determine factors associated with mastitis, and antimicrobial susceptibility profiles of bacteria isolated from mastitic milk in dairy camels in the Benasdir region, Somalia.

1.4.2 Specific Objectives

- To isolate and identify bacteria from clinical and subclinical mastitis cases in dairy camels in Benaadir region, Somalia,
- To establish antimicrobial susceptibility profiles of the isolated bacteria
- To determine the factors associated with occurrence of mastitis in camels in Benaadir region, Somalia.

1.5 Hypothesis/Hypotheses

- ✓ There is a high prevalence of mastitis cases (both clinical and subclinical) in dromedary camels found in Benaadir region, Somalia
- ✓ Bacteria isolated from mastitic cases in Benaadir region, Somalia, are susceptible to antimicrobials
- ✓ Several factors are associated with occurrence of mastitis in Benaadir region, Somalia

CHAPTER TWO: LITERATURE REVIEW

2.1 Camel production in Somalia

Camel (*Camelus dromedarius*) is a domesticated animal that is well adapted to hot arid environments. Among many breeds of camels, the one-humped camel (*Camelus dromedarius*) is mostly domesticated. A large variety of distinct camel breeds that developed along tribal/ethnic lines are due to factors such as specific utilization patterns of individual cultures and purposeful selection for certain criteria. There are recent initiatives for indigenous animal breeds owing to the increasing awareness of genetic resources. Additionally, camel production seems to be of key relevance in the future following the progressive global environmental deterioration (Ahmad *et al.,* 2010).

The population of camels in the world, Africa and Somalia is 37.5, 32.6 and 7.2 million as of 2019 (FAOSTAT, 2019). The camels are distributed in the horn of Africa, the Middle East, South Asia, Maghreb and the Sahel. In the Horn of Africa, camels are mostly distributed in the arid lowlands of Eastern Africa namely, Somalia, Sudan, Ethiopia, Kenya and Djibouti. According to Ornas and Hussain (1993), camels are multipurpose animals that produce milk, meat, hides and skins. Herds of camels are a sign of wealth among pastoralists (ICPALD, 2015). Camel milk is rich in nutrients, minerals and other valuable contents which have made it an acceptable staple food in Somalia. Camel milk has a lower sugar level, low level of cholesterol and higher vitamin C than cow milk, hence a healthy food for people living in arid and semi-arid areas (Mullaicharam, 2014; Ali *et al*., 2016).

The suitable anatomical and physiological characteristics and low susceptibility to diseases of camels make them a potential animal for reliance for its milk and ability to survive harsh arid and semi-arid conditions in sub-Saharan Africa (Scharwtz and Dioli, 1992). As a result, this puts camels among the key animals that can adapt to harsh climatic conditions experienced in sub-

Saharan Africa, for instance, Somalia. The occurrence of mastitis has been a threat to camel milk production and consumption. It has been associated with negative effects such as increased culling, discarded milk, infertility and low yields (Halasa *et al.,* 2007; Seegers *et al.,* 2003). There is little information on mastitis in camels in Somalia, however, there are several cases of mastitis reported in East Africa, the Middle East and Egypt (Moustafa *et al.,* 1987; Karmy, 1990) and Somalia (Arush *et al.,* 1948; Abdurrahman *et al.,* 1992) but none focussed on factors associated with increased prevalence and occurrence of mastitis.

2.2 Causal agents of mastitis in camels

Several bacteria such as *Streptococcus* species, *Staphylococcus* species, *Micrococcus* species, *Aerobacter* species and *Escherichia coli* have been identified as the causal agents of mastitis in camels (Galgalo *et al.,* 2017). According to Woubit *et al.* (2001), mastitis in camels is caused by *Staphylococcus aureus, Escherichia coli, Micrococcus spp*., *Streptococcus agalactiae, Bacillus ovens, Actinomyces,* and *Corynebacterium bovis*. In a study done by Makau (2017), the most isolated bacterium was *Staphylococcus aureus* (61.64%) and was followed by *Streptococcus* species (10.59%), *Pseudomonas* species and *Bacillus* species (6.85% each), *Corynebacterium* species (4.1%) and *Escherichia coli* (at 3.5%). However, in dairy goat mastitis, *Staphylococcus aureus* was the most frequently isolated bacterium (45.34%) (Makau *et al.,* 2017). In addition, there are other bacteria either as a single or mixed infection of mastitis in camel's mammary glands. A study by El-Jakee (1998) reported *Clostridium perfringens* as the causal agent of mastitis in camels, whereas Suheir *et al.* (2005) identified *Mycoplasma arginine*, mould and yeast in mastitic milk of camels. Globally, several studies have been carried out to determine the causal agents of mastitis in camel milk. Besides establishing the camel mastitis causal agent, Galgalo *et al.* (2017) reported acute and chronic prevalence of mastitis among female camels.

The profound changes in dairy farming in most developed countries concomitantly resulted in a major decrease in the prevalence of contagious mastitis and a relative or absolute increase in the incidence of environmental mastitis (Barkema *et al.,* 2015; Klaas and Zadoks, 2017). Environmental mastitis is a disease syndrome with several potential causal agents and contributing factors emanating from the host and the environment (Klaas and Zadoks, 2017). Other studies have shown that mastitis caused by *E. coli* is transient and its disease outcome depends on the host factors such as vitamin deficiency (Smith *et al.,* 1997), lactation stage (Burvenich *et al.,* 2003) and energy balance (Suriyasathaporn *et al.,* 2000).

2.3 Transmission of camel mastitis

Infection of mastitis depends on the concentration of the causal agent in the environment. However, Galgalo *et al.* (2017) revealed three factors affecting camel mastitis. First, efficiency in milking as well as associated hygiene. Second, the susceptibility of the animal/camel to mastitis highly depends on the lactation stage and age. In addition, the level of inherited resistance is partly determined by the teat anatomy. The third is mammary gland immunity. Therefore, the presence of mastitis causal agents, poor hygiene, high susceptibility of the animal/camel and low immunity of the mammary glands contribute to the occurrence of mastitis in camels.

2.4 Economic losses due to mastitis

Halasa *et al.* (2007) reported the direct and indirect economic losses associated with mastitis in livestock. Given the wide host range of mastitis pathogens, yield losses can persist for a month (Hertl *et al.,* 2014a). A study by Cha *et al.,* (2013) showed the association of culling with mastitis in livestock. Makau (2017) showed that mastitis among livestock has the potential to decrease the quantity, quality and milk-based products as a result of discarded milk, early culling, cost of drugs,

veterinary fees and increased labour expenses. A similar study by Lightner *et al*. (1989) on camels showed high leucocyte counts, clots and discolouration in milk upon mastitis infection. Based on symptoms, there is clinical and sub-clinical mastitis characterised by the presence of clots and signs of inflammation in normal-appearing milk, respectively (Abdelgadir, 2014). Furthermore, all cases of mastitis reduce milk production.

2.4. Clinical mastitis

Clinically, mastitis presents side effects such as abnormal milk, swelling of udders, redness, heat, pain and blocked udders which advance to tissue damage and a decrease in milk production (Sundholm, 1995). Thus, the udder is painful, hot and red, with induration. In clinical mastitis, the milk often has discolouration with clots whereas, in high severity, it has clumps of fibrin (Samad, 2022). Dubad *et al*. (2019) revealed elevated temperature, lethargy and anorexia as systemic clinical signs of mastitis. The condition normally occurs as a result of a lack of post and/or pre-milking teat dipping, unhygienic conditions, lack of treatment and poor management. Although clinical mastitis can be diagnosed and treated, prevention and control measures are important. The curative approaches have been associated with poor success and are costly (Hussain *et al*., 2017).

Clinical mastitis is diagnosed through palpation of the udder and the use of a strip cup to check for flakes and clots. Clinical mastitis is further classified as acute, sub-acute and chronic, based on the time course of the disease. For instance, among the characteristics of acute mastitis are inflammation of the mammary gland and abnormal milk. Furthermore, the systemic signs of acute mastitis include shivering, fever, anorexia and depression (Ismail and Dickson, 2010**).**

Chronic mastitis does not show clinical signs for long periods, meaning that the mammary gland remains infected but without symptoms for a long time, then periodically there is the production

of signs similar to those of acute mastitis (as given above) (Aqib *et al.*, 2022). The upsurges of mastitis may be very severe to produce milk containing flakes and shreds of fibrin. According to Ramadan *et al.* (1987), unilateral chronic mastitis in female camels obstructs the teat canal by keratin which leads to the dilation of the teat duct, retention of milk and secondary bacterial infection. Ismail and Dickson (2010) reported *Staphylococcus species* as being responsible for chronic mastitis which causes a reduction in milk production**.**

2.5 Subclinical mastitis

Among the characteristics of subclinical mastitis in camels are infected udder and less milk production. However, the udders are neither swollen nor the milk abnormal. Lack of visible signs of inflammation presents a challenge in the diagnosis of subclinical mastitis but depends on indirect procedures to determine the presence of either immune cells or antibodies in the milk (Galgalo *et al*., 2017*)*. For example, both the California mastitis test (CMT) and Somatic cell count (SCC) can be employed to indirectly determine subclinical mastitis. California mastitis test can be applied in the field; the CMT reagent is a detergent with a pH indicator added (the reason for the purplish colour). Blending equal amounts of milk with the CMT reagents enhances the disruption of the outer cell wall and nuclear cell wall of the leucocytes **(**Mellenberger, 2017). Either white blood cells or leucocytes are dominant somatic cells in milk that confer protection in mammary glands (Ismail and Dickson, 2010). Somatic cell count (SCC), a microscopic technique, requires a laboratory set-up to determine the results (Galgalo *et al.*, 2017).

2.6 . Streptococcal mastitis

Obied and Bagadi (1996) have reported the occurrence of intra-mammary infection in camels due to *Streptococcus agalactiae* and *Staph. aureus.* However, according to Radostits *et al*. (1997), *Staphylococcus aureus* was observed as a frequently occurring bacterial causal agent in dairy

cows, though *Streptococcus agalactiae* was associated with greater production losses. A low prevalence of less than 2% due to streptococcal mastitis in goats was associated with a high somatic cell count (Contreras *et al.*, 1995; Hall, 2007). Streptococcal mastitis is contagious since the organisms are either inside udders or underneath the skin. Furthermore, mastitis is persistent and mostly transmitted through milking (Khan and Khan, 2006).

2.7 Staphylococcal mastitis

Majority of studies on staphylococcal mastitis are in cattle rather than camels. However, Aqib *et al.* (2017) reported a 50% prevalence of subclinical mastitis in camels where staphylococci were cultured in 74% of the tested milk samples. Additionally, there was over 80% prevalence of coagulase-positive *Staphylococcus aureus* whereas that of coagulase-negative *Staphylococcus aureus* was about 50% (Aqib *et al.*, 2017). Although *Staphylococcus aureus* was among the most dominant bacteria isolated from camel milk, Azmi and Hassawa (2008) reported clinical signs of mastitis in about 21% of camels; with bacterial counts ranging from 3.0×10^2 to $< 3.0\times10^3$ CFU per ml in milk samples.

2.8 Coliform mastitis

Coliforms mainly *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter aerogenes* are environmental pathogens responsible for mastitis. On the other hand, *Pasteurella multocida, Pseudomonas* species and *Serratia marcescenus* are among the less-common mastitis-causing pathogens. Additionally, *Escherichia coli, Pasteurella hemolytica, Pseudomonas aeruginosa, Brucella melitensis* and *Staphylococcus aureus* have been identified in mastitic mammary glands of camels (Hegazy *et al.*, 2004). Furthermore, *Pseudomonas aerogenosa* and *Escherichia coli* were mainly isolated from the coagulative necrotic regions with inflammatory zones (Hegazy *et al.*,

2004). Nagah and Thabet (1993) and Bakeer *et al.* (1994) observed similar findings in a cow with acute mastitic *Escherichia coli* infection.

2.9 Mycoplasma mastitis

Mycoplasma organisms are simple bacteria without cell walls and with less genetic code than most bacteria. The organisms have the potential of causing mastitis (Razin *et al.*, 1998). Most *Mycoplasma* mastitis cases are under-diagnosed owing to the organisms' simple genome and fastidious growth associated with slow replication. Suheir *et al.* (2005) identified *Mycoplasma arginine,* besides moulds and yeasts, among mastitic camels in Egypt. Another study by Abo-Elnaga *et al.* (2012) reported a *Mycoplasma* prevalence of 33.9% and 43.3%, as determined by culture and PCR, respectively.

2.10 Environmental factors associated with mastitis

Various environmental factors are directly associated with both clinical and subclinical mastitis. Water, mud, dirt, milkers' hands, manure and bedding are all possible sources of mastitis causative organisms. Environmental pathogens include *Streptococcus uberis*, *Klebsiella pneumoniae* and *Escherichia coli* (Makau. *et al* 2017). The presence of pathogens on teat orifices and the population of bacteria in bedding both influence the rate of mastitis infection (Abunna, *et al.* 2013). The exposure of the mammary gland to pathogenic organisms can be reduced by using a teat cannula to prevent infections from entering the teat orifice or by improving hygiene in the herd (Makau *et al.*, 2017).

Both environmental streptococci and coliforms develop faster in wet and warm temperatures. This increase in humidity and temperature leads to an increase in the number of pathogens in the

bedding (Smith *et al.,* 1985; Makau, 2017). The best bedding should be from organic materials. In addition, the best practice is to wash one's hands before milking (Makau, 2017).

2.11 Epidemiology of clinical and subclinical mastitis

Mastitis is partly associated with herd management and the geographic location of the farm. Mastitis is an important disease with the potential of reducing milk production (Makau, 2017). For instance, there was a 31% prevalence of camel clinical mastitis in Finland and Uruguay by 2001 (Hussain *et al.,* 2017). Haftu *et al.* (2013) reported 29-38% and 10-17% of sub-clinical and clinical mastitis prevalence, respectively, in Southern Ethiopia (Borana area) almost for all seasons in dromedary camel. However, in Sudan, there was a 45% and 1% prevalence of sub-clinical and clinical mastitis, respectively (Haftu *et al.*, 2013). In another study in Sudan, the bacterial isolates were *Staphylococcus* (80%), *Streptococcus* (2%), *Corynebacterium* (3%), *Bacillus* (9%) and *Pasteurella* (6%) (Ismail and Dickson, 2003). In Somalia, the overall mastitis prevalence was established as 23% whereby that of cattle, camel and goats were 27.4%, 25.5% and 16%, respectively (Dubad, 2019).

2.12 Diagnosis of camel mastitis

Though mastitis can be detected either from the mammary gland inflammation or the presence of mastitis pathogen(s), Pyorala *et al.* (2011) used somatic cell count, electrolytes, enzymatic markers or acute phase protein to determine the occurrence of inflammation. As much as pathogen(s) detection is generally based on traditional culturing, Dohoo *et al.* (2011) revealed a range of opinions on culture results interpretation. For example, phenotypic or genotypic methods were used by Zadoks and Watts (2009) to identify the bacterial species. On the contrary, Munoz *et al.* (2007) revealed the unreliability of phenotypic identification of mastitis pathogens based on

biochemical profiles, especially for *Staphylococcus* and *Klebsiella* species. Owing to advanced technology, Cameron *et al.* (2017) used proteomics based on the matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis. Koskinen *et al.* (2009) identified the mastitis pathogen(s) using PCR and sequencing of housekeeping genes on samples of milk as well as cultured isolates, respectively. Surprisingly, Oikonomon *et al.* (2012) detected several mastitis-causing microorganisms in healthy mammary glands. As a result, this calls for one not to rely on phenotypic techniques of detecting mastitis only, but rather supplement with molecular techniques. Among these molecular techniques include loop-mediated isothermal amplification (LAMP) whereby Sheet *et al.* (2016) identified *Staph. aureus* primers and Bosward *et al.* (2016) and Wang and Liu (2015) identified *Streptococcus* primers.

2.12.1 Physical Examination of Udder

Examination of the mammary gland is important for the successful detection of mastitis. Physical examination focuses on the shape, size, consistency and contour of udders to determine inflammation, swelling and loss of function. Varshney (2000) reported that the physical examination of the udder was quite informative (about the size, shape, and consistency) when conducted immediately after milking.

2.12.2 California Mastitis Test (CMT)

California mastitis test (CMT) is an on-farm rapid test for the diagnosis of subclinical udder infections based on the gel formation of deoxyribonucleic acid (DNA) released by somatic cells when lysed by the detergent (Figure 2.1). The scaling of the CMT reaction ranges from 0-3, where 0 denotes no reaction, 3 denotes a strong reaction, while 1 and 2 denote reactions that are in between (Dingwell *et al.,* 2003). Scaled results from CMT were determined by Polat *et al.* (2008) to approximate the somatic cells in milk. Ramadan *et al.* (1987) considered CMT more of an

indicator rather than a definitive test for somatic cells in milk. In addition, irritation of the mammary gland was associated with an increase in somatic cells in milk (Saleh *et al.*, 2011). Therefore, bacterial culture needs to be done on all positive CMT samples to confirm the causative agent (Makau, 2017).

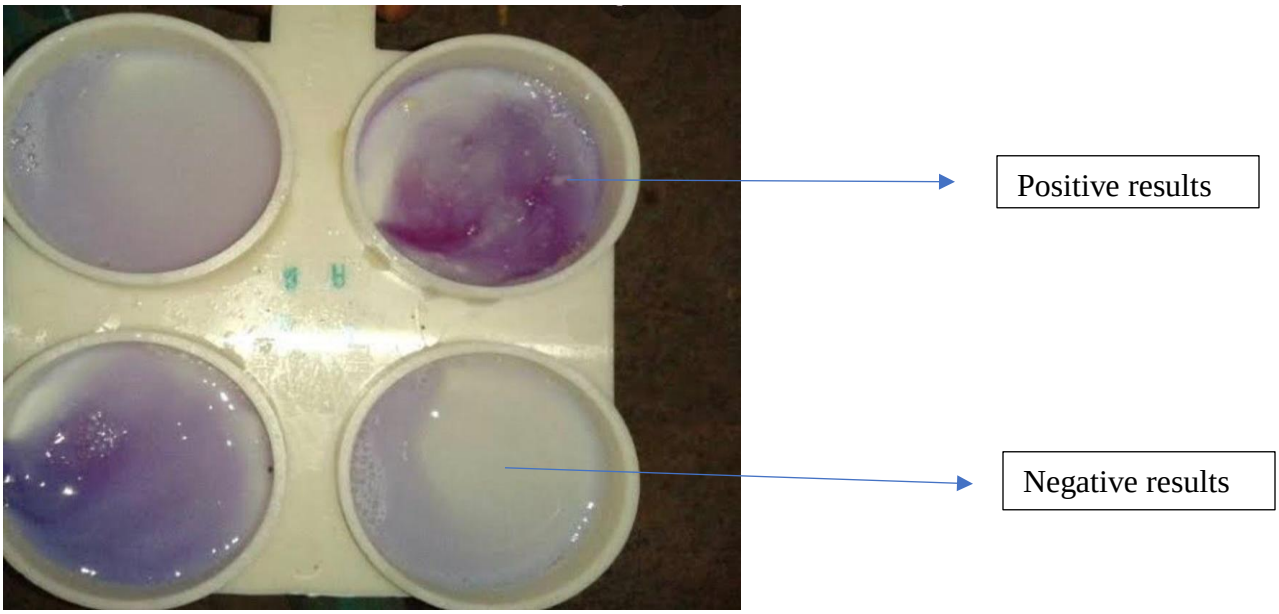

Figure 2. 1: CMT denoting positive and negative results

2.12.3 Somatic Cell Count (SCC)

Somatic Cell Counting is a technique that establishes the number of leucocytes in a milk sample using an automatic cell counter. The samples can either be analysed on the farm using a portable counter or in the laboratory. Alternatively, the samples can be assessed using a direct microscopic somatic cell count (DMSCC) in a laboratory (Saleh *et al.*, 2011).

2.12.4 Electrical conductivity

The electrical conductivity of milk had been considered a screening test for subclinical mastitis (Fernando *et al.*,1982). The electrical conductivity increases during mastitis as sodium (Na) and chloride (Cl-) levels rise and potassium (K) levels fall. These variations in conductivity can be

identified using portable or milk line instruments. The collected data can be analysed using computer software to identify animals with abnormal electrical conductivity (Saleh *et al.,* 2011).

2.12.5 Bacteriological analysis

This is a direct method of diagnosing mastitis through culturing of milk samples for isolation and identification of microorganisms following standard procedures (Makau, 2017). Depending on the organism, media supplemented with either blood agar or MacConkey agar could improve the culturing conditions as well as its identification. Incubation of the culture also depends on the bacterium being isolated, however, upon obtaining a pure culture, identification of the organism is based on respective biochemical tests (Galgalo *et al*, 2017).

2.13 Antibiotic susceptibility test

Antibiotic susceptibility tests determine the effective antibiotic for the treatment of the particular causative agent (Liasi *et al.*, 2009). The disk diffusion method - based on the Mueller Hinton agar - is the common antibiotic susceptibility test method (NCCLS, 2006). Sterile cotton swabs are used to transfer diluted bacterial suspension onto Mueller-Hinton agar plates. The swabs are streaked severally on the whole agar surface to seed the bacteria uniformly. Paper discs impregnated with antibiotics (e.g., ampicillin, sulphonamide, gentamycin, kanamycin, streptomycin, norfloxacin, tetracycline, cotrimoxazole) are applied using sterile forceps onto the streaked surface followed by incubation overnight at 37°C. If the streaked organism is susceptible to a particular antibiotic, a zone of inhibition (no growth) will be seen around the respective antibiotic. The zone diameters are then recorded and the classification of micro-organisms as to whether they are susceptible or resistant to a particular antibiotic is done following the guidelines given in the manual developed by Stephen *et al*. (2005) of the National Committee for Clinical Laboratory Standards (2006). Although the interpretation of the inhibition zones is different for each bacteria-antibiotic

combination, generally an inhibition zone diameter of ≤14mm is scored 'R' for the resistant while an inhibition zone diameter of ≥15mm is scored 'S' for susceptible (NCCLS, 2006).

2.14 Prevention and control of Mastitis

2.14.1 Biosecurity

In response to the prevention of the introduction of mastitis pathogens, initiation of external biosecurity is key. For example, Mweu *et al.* (2014) reported no movement of cattle from *Serratia agalactiae* - positive herds in Denmark. In reducing bacterial exposure, Dohmen *et al.* (2010) and Munoz *et al.,* (2008) reported scoring tools for cow, udder and teat cleanliness ideal in preventing the further introduction of mastitis among herds in the United States. The use of lime was observed to reduce bacterial load counts and damage on teat skin (Kristula *et al.,* 2008; Paduch *et al.,* 2013). However, there is no ideal method of managing livestock beddings to reduce exposure to environmental mastitis pathogens (Leach *et al.,* 2015; Rowbotham and Ruegg, 2016).

2.14.3 Treatment

In the management of mastitis, both hygiene and therapies need to be mutually integrated, for instance, to effectively minimise intra-mammary infection. A study by Philpot (1979) further elaborates on the benefits of hygiene and therapies following their relevance in reducing the frequency of infection and increasing the rate of elimination of the causal agent, respectively (Philpot, 1979).

The milking order should be followed for effective control of environmental mastitis. Camels that are infected should be milked last and feed should be provided to all camels immediately after milking to ensure that they remain standing for thirty minutes to allow the teat orifice to close (Farah, 2004). Culling of chronically infected camels is encouraged, to prevent them from

spreading the disease to others; it is also cheaper to replace them than treat mastitis (Makau *et al*., 2017).

Many livestock species including camels can be infected with mastitis whereby its management is through antibiotics. According to Barlow (2011), the efficiency of an antimicrobial treatment depends on the immunity of the animal, virulence of the causal agent and clinical manifestation. Among the treatments of mastitis include the use of systemic antimicrobials (trimethoprim-sulfamethoxazole, penicillin/aminoglycoside and methicillin), anti-inflammatory drugs (flunixin meglumine), stripping on the mammary glands and hydrotherapy which also partly reduces local oedema (Galgalo *et al.,* 2017*)*. Lenin (2004) used commercial intramammary infusion drugs in the treatment of subclinical mastitis. High doses of systemic antibiotics are used in the treatment of mastitis. Among the antibiotics against mastitis are Oxytetracycline (10mg/kg), Tyrosine (12.5/kg), Penicillin (16500 U/kg), Sulphadimidine (200mg/kg), Erythromycin (10mg/kg), Tylmicosin (10mg/kg), Kanamycin (10mg/kg) and Ampicillin (10mg/kg). Administration of dexamethasone 5mg/kg body weight in the mammary gland has been reported to reduce swelling (Makau, 2017).

Unlike *Klebsiella* and *Strept. dysgalactiae,* there are several studies on antibiotic formulations specific for *Staph. aureus* (Barkema *et al.,* 2006), *Strept. uberis* (Zadoks, 2007) and *E. coli* (Suojala *et al.,* 2013). These fundamental standards can all be achieved following a proper diagnosis and its therapy, supplementation of deficient nutrients and ensuring the livestock hygiene measures. Antibiotic formulations against mastitis have been developed, however, in camels, their efficacies remain elusive (Jimale, 2018). Other studies used internal (Huxley *et al.,* 2002) and external (Lim *et al.,* 2007) teat-sealants and teat-dips (Lopez-Benavides *et al.,* 2009) against mastitis in non-lactating animals.

2.14.4 Vaccination

There is minimal documentation of studies on camel mastitis; however, in other livestock, there is documentation of several attempts of developing vaccines. Though the scope is mostly limited to a few mastitis pathogens such as *E. coli*, *Staph. aureus* and *Strept. uberis*, the vaccines were developed to purposely reduce the disease severity and mortality as well as reduced milk loss (Schukken *et al.*, 2014; Smith *et al.*, 2006). Attempts to develop *Staph. aureus* vaccines date back to the 1960s; however, the resultant products in the market are not satisfactory (Landin *et al.*, 2015). Nevertheless, similar effects in reducing mastitis severity and reduced yield loss upon vaccination were reported by Bradley *et al*. (2015a) and Schukken *et al.* (2011b).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Ethical approval

Ethical approval for this study - for shipment of camel milk samples - was granted by the Biosecurity, Animal use and Ethics committee of the Faculty of Veterinary Medicine, University of Nairobi in Kenya. Considering the study was conducted in Somalia, a permit for conducting the study on camels was granted from Somalia (Appendix 4).

3.2 Study Area

This study was conducted in Benaadir region (2°2′59″N; 45°15′44″E) of Somalia from April 2021 to April 2022). The region borders the middle Shebelle from the North and East, the lower Shebelle from the West and the Indian Ocean from the South. In this region, there are a total of 18 districts but three were purposively selected for this study and include Dharkeynley, Yaaqshid and Huriwaa (Figure 3.1). The region sits on 96,878 km^2 of land with the largest human population of 2.3 million (Wikipedia, 2018).

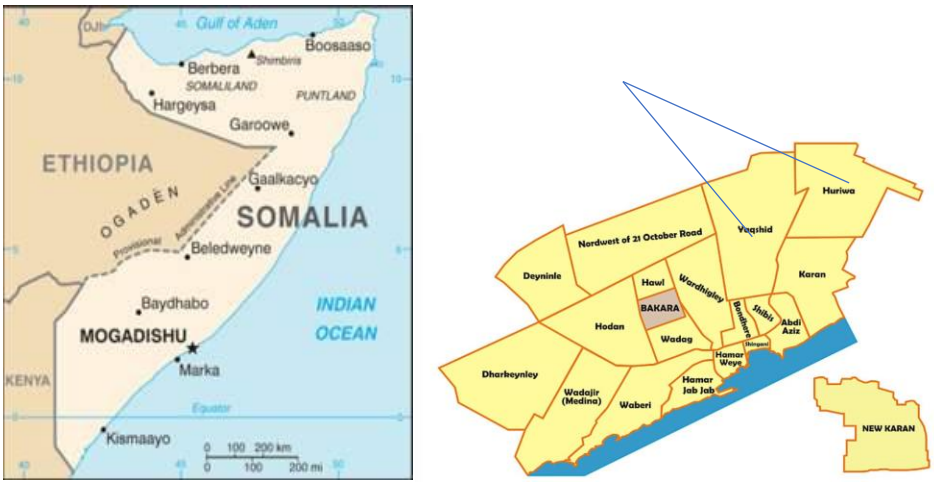

Figure 3.1: Maps showing the location of Somalia in Africa and the Benaadir region

3.3 Study design and sample size

A cross-sectional study was conducted in the Dharkenley, Yaaqshid and Huriwa regions of Benaadir in Somalia to determine the prevalence of mastitis in camels. A total of 290 camels were used across the three purposive selected districts to establish the prevalence of mastitis. The sample size of the camels was computed as given by Dohoo *et al.* (2003);

$$n = \frac{z_a\, p(q)}{L^2}$$

Herein, n= sample size, Z_a = 1.96, p= a prior prevalence (estimated prevalence), q= 1-p and L= allowable error. A prior prevalence used was 25% following a study by Toroitich *et al.* (2017) in West Pokot in Kenya which were similar to production systems in Somalia.

The semi-structured questionnaire was administered to a sample size of 96 herdsmen, parallel with the collection of milk samples among the 290 camels. The number of herdsmen was a third of the number of camels sampled to enable efficiency in gathering data on socioeconomic, camel-rearing practices and hygiene measures during milking. Therefore, in each district - Dharkenley, Yaaqshid and Huriwa – a total of 32 herdsmen were interviewed.

3.4 Milk sample collection

Milk samples were collected directly from the cleaned udders of the restrained female camels early in the morning. The teats were first disinfected using 70% alcohol and allowed to dry. After discarding the first four streams of milk, the California Mastitis Test (CMT) was carried out and then approximately 10 ml of milk samples from each half were collected into labelled sterile tubes. The samples were then transported in frozen conditions to the Department of veterinary pathology, microbiology and parasitology (VPMP), University of Nairobi for bacterial isolation and

identification. Figure 3.2 shows the researcher handling one of the camels before sample collection.

Figure 3. 2: Researcher handling one of the camels before collection of milk sample

3.5 Isolation and identification of bacteria

From the collected milk samples, bacteria were isolated and identified based on the procedure of Galgalo *et al.* (2017). A loop full of the milk sample was streaked onto the respective agar medium (blood agar, MacConkey agar, Mannitol salt agar, etc.). The streaked plates were aerobically incubated at 37°C for 18-24 hours. Isolated pure colonies from the samples were further subjected to primary and secondary biochemical tests for their identification (Makau, 2017) (Appendix 1).

3.6 Determination of antimicrobial susceptibility patterns of bacteria isolated from the camel milk samples

Antimicrobial susceptibility testing was carried-out on the five most isolated bacteria in the study. Culture media were separately incorporated with the following antibiotics: Gentamycin, Ampicillin, Kanamycin, Penicillin, Tetracycline, Norfloxacin, Erythromycin and Streptomycin.

Thereafter, all the cultured media supplemented with respective antibiotics were streaked with the five most isolated bacteria namely *Staphylococcus aureus, Streptococcus spp*., *Enterobacter spp.*, *Citrobacter spp.* and *Escherichia coli*. The inhibition zone of each streaked bacteria was determined after incubation for further classification. Breakpoints based on the CLSI guidelines (2016) were used to classify the bacteria isolates into susceptible, intermediate and resistant. The number of susceptible, intermediate and resistance samples of respective bacterial isolates against the antibiotics tested was converted into a percentage of the total sample size. Cases of antimicrobial resistance, including multi-drug resistance, were noted.

3.7 Collection of data for identification of risk factors

Data was collected from 96 respective camel farmers through the administration of questionnaires as shown in Appendix 2.

3.8 Data handling and analysis

Data were entered in a Microsoft Excel spreadsheet and exported to SPSS version 20 for Statistical analysis. Descriptive statistics were generated using the same statistical package. Differences in proportions were assessed using the Chi-square at a 5% level of significance in univariate analysis. Similarly, in establishing the strength of the association of the dependent variable, "respondents reported mastitis cases among camels", a step-wise binary logistic regression (Enter) was used with the potential independent variables as indicated in Table 4.8. The resultant association were based on the odds ratio.

CHAPTER FOUR: RESULTS

4.1 Prevalence of clinical and subclinical mastitis in dairy camels in Benaadir region

4.1.1 Clinical mastitis: Presence of lesions on teat and udder

Among the 290 sampled camels, 11% (33 camels) were reported to have lesions on the teat and udder. These lesions were characterized by fibrosis (3%), supernumerary teats (2.4%), injuries (3.4%) and oedema (2.4%). All these lesions' characteristics were less than 4%. From the physical examination for clinical mastitis, the prevalence was 1.4% (4 confirmed cases) within Benaadir region of Somalia. Herein, both left and right udder halves of the cases were affected. Figure 4.1 shows a case of oedema on one camel mastitis case.

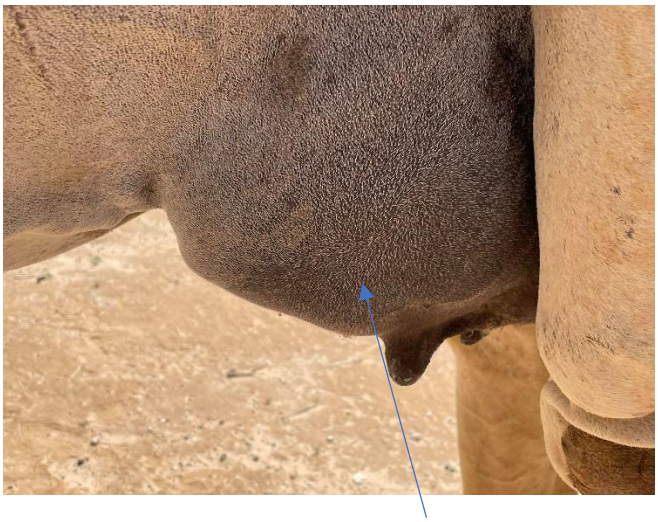

Figure 4.1: A case of oedema on the udder (arrow) of camel

4.1.2 Prevalence of subclinical mastitis based on California Mastitis Test

Two hundred and eighty-six (286) camels had their milk tested for subclinical mastitis, using California Mastitis Test. There was a 29% overall prevalence of subclinical mastitis across the

three districts. However, Huriwa, Dharkenly and Yagshida had a prevalence of 28.1%, 29.5% and 29.5%, respectively. The occurrence of subclinical mastitis was not significantly different within the three districts. Among the camels with subclinical mastitis, both left and right udder halves were affected (Table 4.1).

Table 4.1*:* California Mastitis Test results on milk samples across the three districts in Benadir Region in Somalia

Farms	No. of camels	No. Positive	Prevalence	P-value
Huriwa	96	27	28.1	0.972
Dharkenly	95	28	29.5	
Yaqshid	95	28	29.5	
Total	286	83	29	

4.2 Isolation and characterization of bacteria from milk samples with subclinical mastitis among dairy camels

Table 4.2 shows bacteria that were isolated from the 286 camel milk samples. With respect to overall varieties of isolated bacteria, Huriwa district had the highest at 50%, whereas Dharkenly and Yaqshid had varieties at 26.3% and 27.3%, respectively. Across the study districts, bacteria from genera *Streptococcus* and *Staphylococcus* had significantly ($p<0.05$) higher prevalences than the rest. Low prevalence of *Escherichia*, *Citrobater* and *Enterobacter* genera were reported in Huriwa and Yaqshid districts. Across the three districts, the prevalence of mastitis-causing bacteria was 34.4% (99/286).

Table 4. 2: Bacteria isolated from study camel milk samples, per study site and overall

Bacteria Isolates	Huriwa N=96		Dharkenly N=95		Yaqshid N=95		P-value
	Positive	Prevalence (%)	Positive	Prevalence (%)	Positive	Prevalence. (%)	
Streptococcus spp	27	28.1	21	22.3	12	12.5	0.027*
Staphylococcus spp	16	16.5	4	4.2	10	10.5	0.015*
Escherichia coli	3	3.1	0	0	1	1.1	0.325
Citrobacter spp	1	1	0	0	1	1	0.44
Enterobacteri spp	1	1	0	0	2	0.7	1
Total	48	50	25	26.3	26	27.3	

* Denotes significant difference between regions and bacteria isolates. Positive number with numbers >5 Chi-Square test was applied while those with <5 Fisher Exact test was applied respectively.

4.3 Antimicrobial susceptibility profiles of bacteria isolated from the camel milk samples

4.3.1 General antimicrobial susceptibility patterns of the tested bacterial isolates

Antimicrobial susceptibility results of the five most-isolated bacteria: *Staphylococcus aureus*, *Streptococcus spp*., *Enterobacter spp*, *Citrobacter spp* and *Escherichia coli* were as given in Figure 4.2 and Appendix 3. The isolates exhibited variable susceptibilities to the 8 tested antimicrobials. All the organisms were susceptible to Gentamycin. All *Enterobacter* and *Citrobacter* organisms were susceptible to all antimicrobials tested. *Staphylococcus* had strains that were resistant to Ampicillin, Penicillin and Streptomycin at 42.3%, 32.6% and 42,3%, respectively. *Escherichia coli* organisms were 100% resistant to Penicillin, while 100% susceptible to Gentamycin, Ampicillin, Kanamycin and Norfloxacin. Unfortunately, some of the tests could not be interpreted as cut-offs were not indicated in the CLSI (2016) table.

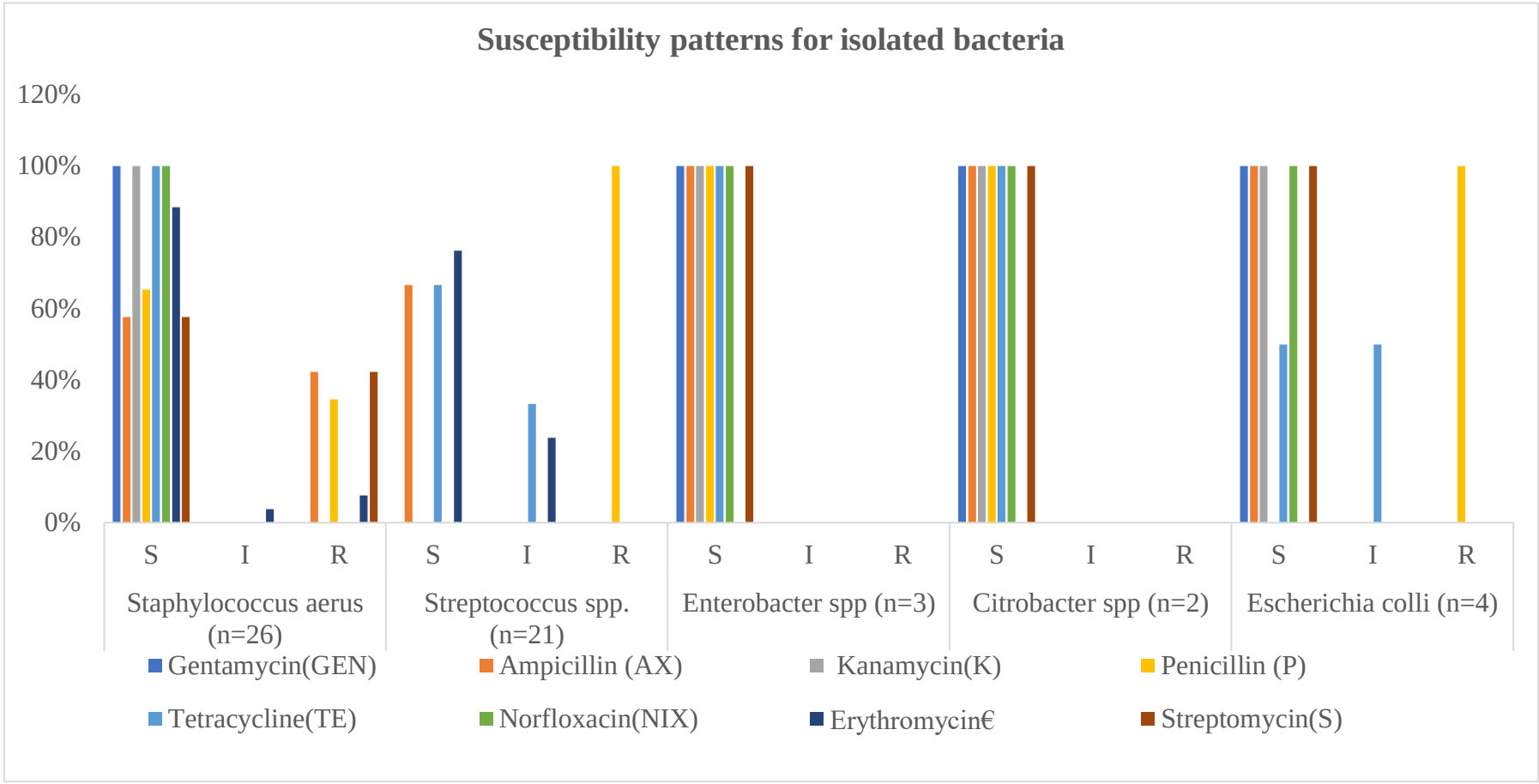

Figure 4. 2: Graphical presentation of antimicrobial susceptibility patterns of most-isolated bacteria from the study milk sample

4.3.2 Multi-drug resistance patterns of the tested bacterial isolates

Multi-drug resistance patterns of the tested isolates were as given in Table 4.3. *Staphylococcus aureus* had 244 isolates that were multi-drug-resistant (66 were resistant to 2; 178 were resistant to 3) – the most common combination was AX/STP/ERT at 9.8%; *Streptococcus* had 189 that were multi-drug-resistant (84 were resistant to 2; 105 were resistant to 3) – all at the same rate of 11.1%; *Escherichia coli* had 36 that were multidrug-resistant (16 that were resistant to 2; 20 that were resistant to 3) – all at the same rate of 11.1%. All tested *Enterobacter* and *Citrobacter* organisms were sensitive to all the tested antimicrobials.

Table 4. 3: Multidrug resistance patterns

Antibiotics	*Staph. aureus* (n=244)	*Enterobacter* (n=0)	*Streptococcus* spp (n=189)	*Citrobacter* spp (n=0)	*Escherichia coli* (n=36)
AX/PEN	2 (0.82%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
AX/ERT	2 (0.82%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
AX/STP	22 (9.02%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PEN/ERT	11 (4.51%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
PEN/STP	20 (8.20%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
PEN/TET	9 (3.69%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
AX/PEN/ERT	22 (9.02%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
AX/STP/ERT	24 (9.84%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
STP/PEN/ERT	22 (9.02%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
GEN/PEN/STP	20 (8.20%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
GEN/ERT/STP	13 (5.33%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
GEN/AX/STP	22 (9.02%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
GEN/PEN/ERT	11 (4.51%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
GEN/AX/ERT	13 (5.33%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
GEN/PEN/AX	20 (8.20%)	0 (0.0%)	21 (11.11%)	0 (0.0%)	4 (11.11%)
GEN/TET/AX	11 (4.51%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

X=Ampicillin, PEN=Penicillin, ERT=Erythromycin, STP=Streptomycin, TET=Tetracycline, GEN=Gentamycin.

4.4 Interpretation of questionnaire data

4.4.1 Determination of factors associated with occurrence of mastitis in camels in Benaadir region, Somalia

4.4.1.1 Socio-demographic characteristics of respondents

Tables 4.4 and 4.5 show the distribution of respondents and their socio-economic characteristics, respectively. This study included 96 respondents, where Huriwa, Dharkenly and Yaqshida had 34.4%, 35.4% and 30.2%, respectively. There was no significant difference between the number of men and women recruited in the study locations (p-value=0.732), indicating that, as far as gender was concerned, both males and females were equally recruited. However, there was a disparity in the age and education level of the farmers within the three locations; a majority of camel farmers (39.6%) were in the age group of 21-30 years (significant p=0.000); the majority of the farmers (54.2%) having no formal education (significant; p=0.002). Concerning social economic characteristics, using the number of camels as an indicator, the majority (75%) kept 26-50 camels (significant; p=0.000).

Table 4. 4: Number of farmers recruited per location

Location	No. of Farmers	Proportion (%)
Huriwa	33	34.4
Dharkely	34	35.4
Yaqshid	29	30.2
Total	96	100

Table 4. 5: Socio-demographic characteristics of the sampled farmers

	Location				
	Huriwa	**Dharkely**	**Yaqshid**	**Total**	**p-value**
No. of farmers	33 (34.4%)	34 (45.4%)	29 (30.2%)	96 (100.0)	0.804
	Gender				
Males	27 (33.3%)	28 (34.6)	26 (32.1%)	81 (84.4)	0.732
Females	6 (40.0%)	6 (40.0%)	3 (20.0%)	15 (15.6%)	
	Age (Years)				
15-20	0	1 (100.0%)	0	1 (1.0%)	
21-30	18 (47.4%)	14 (36.8%)	6 (15.8%)	38 (39.6%)	
31-40	7 (20.0%)	7 (20.0%)	21 (60.0)	35 (36.5%)	0
>40	8 (40.4%)	8 (44.4%)	2 (11.1%)	18 (18.8%)	
DNK	0	4 (100.0%)	0	4 (4.2%)	
	Education Level				
No formal Education	14 (26.9)	17 (32.7%)	21 (40.4%)	52 (54.2%)	
Primary Level	18 (52.9%)	8 (23.5%)	8 (23.5%)	34 (35.4)	
Secondary level	1 (12.5%)	7 (87.5%)	0	8 (8.5%)	0.002
Tertiary level	0	2 (5.9%)	0	2 (2.1%)	
	Number of camels				
15-25	6 (37.5%)	10 (62.5%)	0	16 (16.7%)	
26-50	24 (33.3%)	19 (26.4%)	29 (40.3%)	72 (75.0%)	0
>50	3 (37.5%)	5 (62.5%)	0	8 (8.3%)	

4.4.1.2 Types of farming system practised

Across the study area, there were three farming systems namely; extensive, semi-extensive and intensive, as shown in Figure 4.3. The extensive camel farming system is dominant in Dharkenly and Yaqshid as shown by 46.7% of respondents. On the other hand, the extensive camel farming system was least practised by 6.7% of respondents in Huriwa district. Semi-intensive and intensive camel farming systems were practised at 33.3% each. However, the highest semi-intensive camel farming system was in Huriwa and was followed by Dharkenly and Yaqshida, as reported by 41.9%, 32.3% and 25.8% of the respondents, respectively. Intensive camel farming system was reported as the highest by 36.8% of the respondents in Dharkenly whereas both Huriwa and Yaqshida; each had 31.6% of responses.

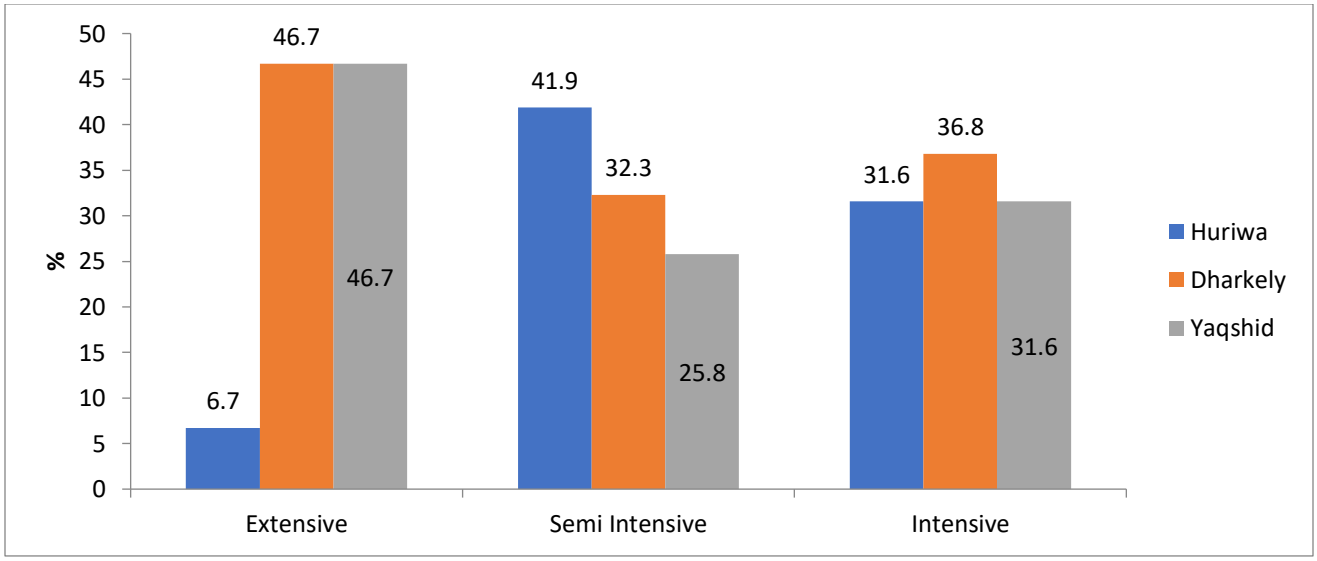

Figure 4. 3: Camel farming systems practised in the study area

4.4.1.3 Source of water for the camels

The sources of water for camels in the three districts were as shown in Table 4.6. There was no significant difference across the three study districts in responses on the sources of water for the camels. Among the water sources, the use of tap water was reported by 54.2% and was followed by that of water pan or flood water. Providing camels with water from either local rivers/streams or local wells/boreholes had the least responses, each of less than 10%.

Table 4.6. Sources of water for camels in the three districts in Benaadir region of Somalia

Water source for camels	Location				
	Huriwa	Dhakenly	Yaqshid	Total	p-value
Tap Water	12 (23.1%)	14 (26.9%)	26 (50.0%)	52 (54.2%)	
Water pan/ flood water	17 (53.1%)	14 (43.8%)	1 (3.1%)	32 (33.3%)	0.106
Local river/stream	3 (37.5%)	4 (50.0%)	1 (12.5%)	8 (8.3%)	
Local wells/ boreholes	1 (25.0%)	2 (50.0%)%)	1 (25.0%)	4 (4.2%)	

4.4.2 Determination of Knowledge Attitude and Practices (KAP) contributing to the prevalence of mastitis in Camels

4.4.2.1 Knowledge of mastitis

Responses on the knowledge of camel mastitis in the three study districts of Benaadir region of Somalia were as shown in Figure 4.4. From the results, over 90% of the respondents had knowledge of mastitis in camels across the three districts of Benaadir region of Somalia.

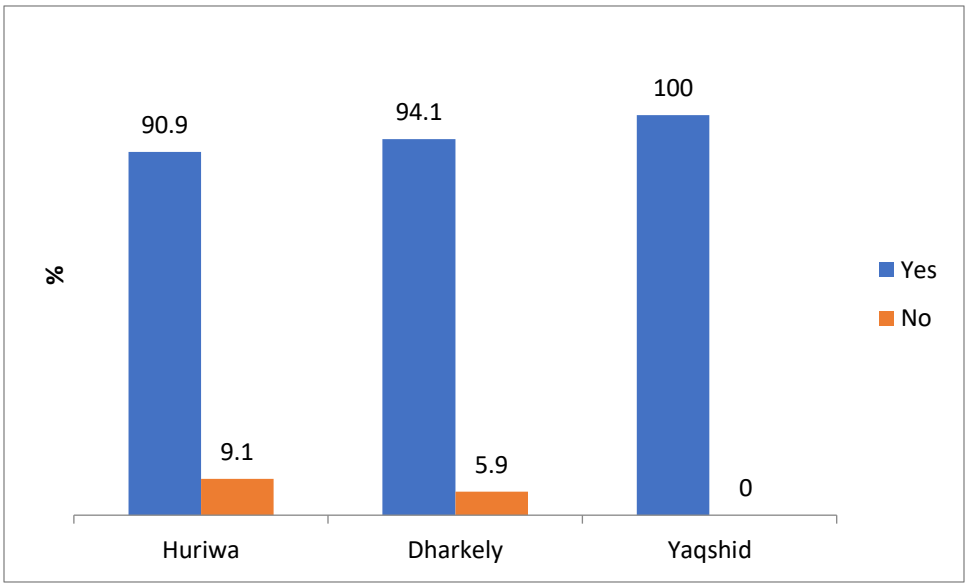

Figure 4. 4: Responses on the knowledge of camel mastitis in the three study districts of Benaadir region of Somalia

4.4.2.2 Period when farmers observed cases of mastitis

The period when mastitis was observed by residents of the three study locations was as given in Table 4.5. In Huriwa and Dharkenly, most respondents had observed mastitis throughout the lactating period, as noted by 70% and 59% of respondents, respectively. In Yaqshid, the majority of 62.1% of respondents observed mastitis in the first three months of lactation. The occurrence of camel mastitis significantly differed in responses between the different lactation periods. However, it needs to be noted that when respondents were further observed, the interviewer noted that only 54.9% of the respondents knew when to observe mastitis correctly, i.e. during the lactating period; the rest (45.1%) knew about mastitis but were observing incorrectly and this was statistically significant (p=0.014), using Fisher exact test.

Table 4. 6: Period when farmers observed cases of mastitis

Location	The first 3 months of lactation	During the whole lactating period	During the dry period	p-value
Huriwa	6 (20.0%)	21 (70.0%)	3 (10.0)	
Dharkenly	10 (31.2%)	19 (59.4%)	3 (9.4%)	
Yaqshid	18 (62.1%)	10 (34.5%)	1 (3.4%)	0.014

4.4.2.3 Milking Hygiene and other Practices

Data on milking hygiene and other practices were given in Table 4.6. The majority of the respondents (37.5%) washed their udder and used an udder towel, while a few (6.25) applied dry matter therapy, respectively. There was a significant difference (p=0.0027) between milking hygiene and other practices. The majority of the respondents milked camels at least twice a day while a few milk at least once a day - in the evening. The majority of respondents (79.2%) were knowledgeable about testing for mastitis while 20.8% of them did not know. The majority (64.5%) of respondents preferred the California mastitis test (CMT) compared to the strip cup method which had 14.5% responses. A significantly higher ($p<0.05$) proportion (52.7%) of farmers engaged a veterinarian in treating mastitis than those that did not (self-medicated).

Table 4. 7: Data on Milking Hygiene and other practices

Milking Hygiene Practices						
Location	Use of teat dips	Wash udder and use of udder towel	Early treatment of new cases	Apply dry therapy	Total	p-value
Huriwa	12 (36.4%)	13 (39.4%)	7 (21.2%)	1 (3.0%)	33 (34.4%)	0.027
Dharkenly	12 (35.3%)	9 (26.5%)	9 (26.5%)	4 (11.8%)	34 (35.4%)	
Yaqshid	2 (6.9%)	14 (48.3%)	12 (41.4%)	1 (3.4%)	29 (30.2%)	
Total	26 (27.1%)	36 (37.5%)	28 (29.2%)	6 (6.2%)	96 (100.0%)	

Location	Methods of testing Mastitis				
	Strip cup	California mastitis test	Test paper	Total	p-value
Huriwa	3 (9.1%)	25 (75.8%)	5 (15.2%)	33 (43.4%)	0.01
Dharkenly	8 (23.5%)	21 (61.8%)	5 (14.7%)	34 (44.7%)	
Yaqshid	0	3 (33.3%)	6 (66.7%)	9 (11.8%)	
Total	11 (14.5%)	49 (64.5%)	16 (21.1%)	76 (100.0)	

Location	Methods of Treating Mastitis					Total	p-value
	Seek Veterinarian	Self-treatment	Antibiotics	Multinjection	Anti-inflammatory drug		
Huriwa	13 (43.3%)	6 (20.0%)	4 (13.3%)	6 (20.0%)	1 (3.3%)	30 (33.0)	0.001
Dharkenly	24 (75.0%)	6 (18.8%)	0	2 (6.2%)	0	32(35.2%)	
Yaqshid	11 (37.9%)	7 (24.1%)	10 (34.5%)	1 (3.4%)	0	29 (31.9)	
Total	48 (52.7%)	19 (20.9%)	14 (15.4%)	9 (9.9%)	1 (1.1%)	91(100.0)	

4.4.3 Summing-up of risk factors, knowledge, attitude and practices, with respect to mastitis in the study camels

These were as given in Table 4.9. The incidence of mastitis was highest (54.2%) among farmers with no formal education and lowest (2.2%) among farmers with tertiary education; however, there was no significant difference (p-value=0.675) between the two. This indicates that education was not a risk factor concerning the incidence of mastitis in the study population. For knowledge about mastitis and its testing, the majority of the respondents (94.8%) knew while 5.2% did not know. The difference between the two was statistically significant (p=0.045), indicating an association between knowledge of mastitis and the method of testing mastitis. Thirty-five-point-five percentage (35.5%) of the respondents treated mastitis by washing the udder with a towel, followed by early treatment (29.2%), while 10.4% applied dry therapy. There was no significant difference between different times of treatment and prevention (p=0.471)

Table 4. 8: Identified risk factors associated with mastitis in the study population

Incident of mastitis	Education Level					
	No formal education	**Primary level**	**Secondary Level**	**Teritial level**	**Total**	**p-value**
The first 3 months	55.60%	33.30%	5.60%	5.60%	37.50%	
During the whole lactation	33.80%	36.50%	9.60%	0	54.20%	0.675
During the Dry period	50%	37.50%	12.50%	0	8.30%	
Total	54.2	35.4	8.3	2.2	100	

Method	Knowledge of Mastitis		Total	p-value
	Knowledgeable	Not knowledgeable		
Strip cup	81.8	18.2	11.5	0.045
California mastitis test	94.4	5.6	56.2	
Mastitis test paper	34.1	100	32.3	
Total	94.8	5.2	100	

Treatment of mastitis	**Prevention**					
	Use of teat dips	**Wash udder and udder towels**	**Early treatment of new cases**	**Apply dry therapy**	**Total**	**p-value**
Seek for Veterinarian	35.40%	25%	29.20%	10.40%	50%	0.471
Treatment by self	23.80%	42.90%	28.60%	4.80%	29.10%	
Antibiotic penistreptomycine	20%	53.30%	26.70%	0	15.60%	
Multi injection	9.10%	54.50%	36.40%	0	11.50%	
Anti-inflammatory Drug	0	100	0	0	1%	
Total	27.10%	35.50%	29.20%	6.20%	100	

Among the potential risk factors associated with mastitis in the study, only the farming systems used were significantly different ($p<0.05$). However, the other independent variables that did not have a significant difference as the risk factors of mastitis in camels were; level of education, number of camels, the water source for the animals, frequency of milking the camels, milking techniques, person frequently milking the camels and uses of milk from the camels. As a result, it was found that, by adjusting these other independent variables (those not showing a significant difference), a farmer can reduce cases of mastitis among his/her camels considerably [Exp (B)= 27.28; 95% CI: 1.360, 547.08; p=0.031] (Table 4.8). Nevertheless, there was no significant difference in the respondents' distribution in terms of the three farming systems used – extensive, semi-intensive and intensive. Rather, the majority (65%) of respondents practising semi-intensive farming systems reported mastitis in camels, whereas both intensive and extensive farming systems had less than 20% each (Tables 4.10 and 4.11).

Table 4. 9: Risk factors associated with mastitis in camels in the study areas

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Level of education	1.85	1.13	2.71	1	.100	6.37	.701	57.87
	Number of camels	1.20	1.16	1.06	1	.302	3.32	.340	32.45
	Farming system	3.31	1.53	4.67	1	.031*	27.28	1.360	547.08
	Water source for feeding animals	.43	.71	.37	1	.545	1.53	.384	6.12
	Frequency of milking the camels	-.72	.94	.59	1	.441	.49	.077	3.05
	Milking techniques	-.00	1.66	.00	1	.999	1.00	.038	25.98
	The person frequently milking the camels	-2.22	1.17	3.60	1	.058	.11	.011	1.08
	Uses of milk from the camels	1.80	1.37	1.73	1	.189	6.02	.413	87.55
	Constant	-15.41	6.15	6.28	1	.012	.000		

a. Variable(s) entered on step 1: Level of education (**1**. Nonformal, **2**. Primary, **3**. Secondary, **4**. Tertiary), Number of camels (**1**. 15-25, **2**. > 25-< 50, **3**. > 50), Farming system (**1**. Extensive, **2**. Semi-intensive, **3**. Intensive), Water source for feeding animals (**1**.Tap, **2**. Water pans/flood water, **3**. Local rivers/streams, **4**. Local boreholes) Frequency of milking the camels (**1**. Once in the morning, **2**. In the evening, **3**. Twice), Milking techniques [**1**. Stripping (pulling the teats), **2.** Squeezing action], Person frequently milking the camels (**1**. Myself, **2**. Family members, **3**. External employs), and uses of milk from the camels (**1**. Family members, **2**. Sale to milk vendors/traders, **3**. Sale to milk collection centres, **4.** Sale to neighbours and members of the community). Asterisk (*) denotes a significant difference at 5%.

Table 4. 10: Response of mastitis based on the camels farming systems

Farming System	Reported mastitis				
	Yes	No	Total	χ^2	p-value
Extensive	15 (100%)	0 (0%)	15 (15.62%)	5.63	0.06
Semi-intensive	60 (96.8%)	2 (3.2%)	62 (64.58%)		
Intensive	16 (84.2%)	3 (15.8%)	19 (19.79%)		

CHAPTER FIVE: GENERAL DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 Socio-demographic Characteristics of Farmers

According to the USAID report of 2019, Somalia is one of the largest homes for the camel population with about 60% of the inhabitants relying on the camel and other livestock for their livelihood. This finding was supported by Wanyoike *et al.* (2015) who established that 65% of the Somalis utilize the livestock industry for income generation. While cattle keeping is regarded as the ideal economic activity for the region, it is not doing well due to the unsuitability of the land for agricultural production (Hand & Kaiser, 2018) - climatic variation affects water availability for livestock sustainability. This situation is buttressed by the findings from this study which established that there is no specific identifiable source of water, as camel farmers had variegated water sources ranging from taps, water pans, local rivers, and local wells. In the study region, there is no significant difference between the two factors (climate variability and water source). For economic activity, the gender representation of camel farmers, as indicated by the current study, is evenly represented; there is also no relationship found to exist between the farmers' gender and the study location. This seems to be the case for the whole livestock sector in the country as both males and females take part in cattle rearing at household levels (Mohamud *et al.*, 2021). Literacy level which plays an important role in livestock care remains to be a bottleneck in the industry. This study confirmed that the education level among farmers in the study area is extremely low; on average, 54.2% of the camel farmers had not attained formal education. This was, however, an improvement from an earlier study which indicated that over 77% of household heads among livestock farmers did not attain any formal education at all (Wanyoike *et al*., 2015). Apart from the gender distribution, the study indicated that the majority of the camel farmers were

young people, between the ages of 21 and 30 years. This may be regarded as a positive step towards achieving self-sustainability among the youth in the country. Additionally, internationally recognized youth strengthening programs such as *Feed the Future, Youth power* and USAID which encourage youth employment to increase youth earnings, have had an impact towards getting young people into the agriculture and livestock sector (Agency *et al.*, 2020). Due to the new trends in science and technology, incorporating the young generation is likely to encourage introduction of science and technology in disease management, increased productivity, and livestock trade which will consequently revolutionize the sector.

5.2 Knowledge about mastitis and its risk factors

Although camels are multipurpose animals, the popularity of this livestock species lies in their milk production aspect. Many camel-keeping communities in Somalia consume camel milk raw without heat treatment (Mohamud *et al*., 2021). This practice can be detrimental to human health if the camel is infected by some diseases. Despite the hardiness of camels to diseases, dromedary camels can be affected by diseases such as mastitis (Geresu *et al*., 2021). Clinical mastitis in camels can be easily identified by camel keepers. However, sub-clinical mastitis can only be identified using diagnostic tests such as California Mastitis Test (Seligsohn *et al.*, 2020). Understanding the occurrence of this disease not only helps in its chemo prophylactic approach, but it also protects the health of consumers. A review of the knowledge base about the disease among camel farmers in the study region revealed that there was a significant difference between the farmers who were aware of the disease and those who were not. A further segmented analysis showed that out of the three study regions, farmers from Dharkely were more knowledgeable of mastitis than their counterparts in Yaqshid and Huriwa. Udder health is an important factor that could influence the development of mastitis in camels. Therefore, hygienic practices such as washing the udder before

milking is essential in preventing udder infection (Seligsohn *et al.*, 2020). These factors are in alignment with findings by Mureithi and Njuguna (2016) which pointed out that breed, stage of lactation, udder hygiene, floor type and parity had a significant influence on the prevalence of subclinical mastitis among dairy cows. These findings were supported by Zeryehun and Abera (2017) and Baraki *et al*. (2021) who carried out studies in Ethiopia. Of this, scrutiny of farmers' practices was carried out. The majority of the farmers were aware of the implications of a lack of proper hygienic practices. Consequently, a large percentage of them reported washing the camel's udder using udder towels. Some of the minority groups who did not practice the above-mentioned hygienic practice opted to apply for hygienic camel housing to maintain the camel's udder health. Other common therapeutic practices that aimed at keeping the camel udder healthy included regular milking, the use of teat dips, and early treatment of new cases. Keeping camel udders empty through regular milking plays a key role in prolonging peak milk production by about 7 to 12% (Waterman *et al*., 2013). As a result, the majority of the farmers who took part in this survey reported milking their camels at least twice daily. Following the fact that subclinical mastitis is common among dairy cattle and that the California Mastitis Test (CMT) is the mostly-used test for its diagnosis, it is not surprising to find that more than three-quarters of the interviewed camel farmers were well-informed on subclinical mastitis and the various tests that can be used. More than half of the interviewees preferred to use CMT. This outcome may be associated with the rapid diagnostic nature of the test (Mellenberger, 2001). The strip cup test which was the least preferred of all tests is not very reliable in diagnosing sub-clinical mastitis (Heider, 2013).

Medical therapy on diseased camels is a common practice among camel keepers. Most farmers turn to veterinary specialists for the treatment of their livestock. According to the Food and Agriculture Organization of the United Nations, professional veterinary services not only aim at

treating sick animals but also offer preventive health services. However, despite these positive aspects, the cost of affording services from a veterinarian remains a big challenge that calls for mitigation (Heffernan and Misturelli, 2001). As a result, other camel farmers employ the use of self-prescribed antibiotics, multi-injection, and use of anti-inflammatory drugs to treat mastitis.

5.2.1 Association between education level and Handling of Mastitis

An evaluation of the relationship between farmers' level of education and identification of the first signs of mastitis showed that the level of education did not play a role in identifying the first signs of the disease. This is because the majority of the farmers who identified the disease within the first three months had no formal education. Additionally, more than half of the farmer population were able to identify the diseased animals by themselves without the aid of a veterinary professional. In a rural set-up, some camel farmers used traditional healing and preventive practices for their herds. For example, they claimed to be able to diagnose a disease by a particular smell of the sick animal (Abdurahman & Bornstein,2011). Although unsubstantiated, these methods have been reported to work over the years (Hashim *et al*., 2009). Extensive knowledge of mastitis among camel keepers, however, has established a linear association with their choices of sub-clinical mastitis test. As opposed to the general finding that most farmers in the study group preferred the California Mastitis Test, the majority of those who were not knowledgeable about mastitis preferred the strip cup test. An evaluation of the prevalence of mastitis revealed that the cases of mastitis are more among farmers with no formal education compared to farmers who had attained minimum formal education. This finding brings into context the role of animal welfare, education and practice. Animal health is an important part of animal welfare can only be enhanced through training of farmers. For instance, Broom (2005) showed that training on animal welfare were effective on literate herdsmen.

5.3 Prevalence of mastitis

During the study, screening for mastitis was done using different types of tests. The numbers were quantified in relation to the specific diagnostic tool.

5.3.1 Based on California Mastitis Test

The number of cases diagnosed by the California Mastitis Test was 29% of the total camel population. This prevalence was close but relatively higher than that of 22.78% reported by Mohamud *et al.,* (2020). Furthermore, the findings from the above-mentioned study were higher than the initial findings by Bekele and Mola (2001); they reported a prevalence of 15.8%. This trend deduces that there is an increasing pattern in the number of camel infections. Cases of clinical and sub-clinical mastitis in animals are not found in Somalia only. Surveys conducted in neighbouring lower Ethiopia showed that the infections are at bay with those identified in Benaadir at 22.4% (Geresu *et al.*, 2021). Adane *et al*., (2017) established that the prevalence of bovine mastitis in and around Jigjiga town- Ethiopia was 9.1%; of which 7.3% were subclinical, 1.8% were clinical mastitis cases. In the Kenya dairy sector, California mastitis test and clinical examination detected 80% of cases of cattle mastitis, out of which 6.8% was clinical mastitis while 73.1% was subclinical mastitis (Mbindyo *et al.*, 2020). The same study asserted that cultural practices at the farm level led to a rise in the prevalence of clinical and subclinical mastitis by 76.6%. Recently, it was confirmed that the prevalence of subclinical mastitis in camels in Kenya was 46% (Dinah, 2021). In Uganda, overall, subclinical mastitis and clinical mastitis cases were at 16.7% and 4.7% respectively. Occurrence and sub-clinical mastitis were responsible for 78% of all the mastitis cases in the country (Zirintunda *et al*., 2017). Mastitic studies on camels conducted in Tumbool -Sudan indicated that 29.7% of female camels had mastitis (Camelus & Abattoir,

2018). Apart from healthcare practices by farmers, other environmental factors are considered to cause a variation in the progression of the disease (Mohamud *et al*., 2020).

5.3.2 Based on bacterial isolates

Bacteriological analysis of the agents of sub-clinical mastitis revealed that Gram-positive bacteria were the major causative agents for the sub-clinical mastitis. These findings are in agreement with previous studies by Zeryehun *et al*. (2013). Furthermore, Mbindyo *et al.* (2020), reported that clinical and subclinical mastitic cases in camels in Kenya were attributed to coagulase-negative *Staphylococcus* (42.8%), *Streptococcus* species (22.2%), *Staphylococcus aureu*s (15.7%), *Pseudomonas aeruginosa* (5.1%), and *Enterobacter* species (0.7%). In their bacteriological and pathological work on camels in Sudan, Camelus and Abattoir (2018) isolated the following bacteria; *Staphylococcus spp* (38%), *Streptococcus spp* (27.6%), *Micrococcus spp* (10.5%), *Corynebacterium spp* (4.8%), *Enterococcus spp* (4.8%), and *E. coli* (2.9%). The outcomes may be associated with the bactericidal nature of the camel udder secretions. The predominant microorganisms isolated in the current study include; *Streptococcus spp, Staphylococcus spp, Escherichia coli spp, Citrobacter spp*, and *Enterobacter spp,* in a declining order. Radostitis *et al*. (2007) affiliated the predominant state of *Streptococcus spp* bacteria to its ability to survive in the camel udder conditions. In addition, the bacterium has a tendency to establish mild sub-clinical infections for a long period of time and consequently can be transmitted to other healthy animals during milking. The presence of the other bacterial species in the camel udder, as studied in this research, have been supported by other studies such as that of Mekbib *et al*., (2010).

5.4 Antimicrobial susceptibility of the bacterial isolates

The prevalence of antimicrobial resistance in dairy products has been on the rise; the case of bacteria in camel milk not being exceptional (Adesetan *et al*., 2013). Bacteria isolated in this study were tested for antimicrobial susceptibility to determine the drug's effectiveness in treating sub-clinical and clinical mastitis. Findings asserted that out of the five isolated species only *Staphylococcu*s *aureus, Streptococcus spp*., and *Escherichia coli* exhibited antimicrobial resistance to the tested antimicrobials. This attribute was an extrapolation of their predominance level during isolation. The resistance of Gram-positive bacteria to Erythromycin and Streptomycin is supported by Adesetan *et al*. (2013). In their case, Srinu *et al*. (2012) found out that *Staphylococcus aureus* was resistant to Pefloxacin but susceptible to Ciprofloxacin, Streptomycin, Cefuroxime and Ceftriaxone. The antimicrobial susceptibility test done on the five isolated bacterial types, in this study, produced a matching outcome with the above-mentioned study because all the bacteria were susceptible to all tested antibiotics except for Erythromycin. Species of *Enterobacter spp*, and *Citrobacter spp*, in particular, showed 100% susceptibility levels. The inability to test for Erythromycin in this study was because its cut-off for *Enterobacter* spp, *Citrobacte*r spp and *Escherichia coli* was not provided in the CLSI (2016). This was the same observation in a similar test study by Amir (2013). An evaluation of multi-drug resistance indicated that *Staphylococcus aureus, Streptococcus spp*., and *Escherichia coli* had multi-drug-resistant strains. A similar study conducted among dairy cattle in Ethiopia indicated a higher rate of multi-drug resistance patterns for *Escherichia coli* bacteria towards Amoxicillin, Penicillin, Gentamycin and Kanamycin (Asamenew *et al.*, 2013). Furthermore, Abera *et al* (2010) discovered that although *Staphylococcus aureus* was 100% susceptible to Gentamycin, Kanamycin, Tetracycline and Norfloxacin, it was resistant to Penicillin. This was similar to what was found in

this study, with some significant levels of susceptibility observed to Erythromycin. However, the resistance level is not significant as findings from the current study recorded a 1% resistance. *Enterobacter spp* and *Citrobacter spp* had no multi-drug resistant cases since the isolates were 100% susceptible to all the tested antimicrobials. This information is comparable to a previous report by Kemal *et al*. (2017).

5.5 Conclusions

- This study revealed that clinical and sub-clinical camel mastitis cases are prevalent in Benaadir. Moreover, progressive studies indicate that these cases are likely to rise even more if rapid mitigating measures are not put in place.
- Udder hygiene practices, sub-clinical diagnostic measures, and environmental factors were identified to be the pre-disposing factors that determine disease progression. Generally, sub-clinical and clinical mastitis was a result of udder infection with *Streptococcus spp, Staphylococcus spp,* and *Escherichia coli spp.* Camel udder condition was observed to be the key factor leading to the survival of the Gram-negative bacteria.
- Total susceptibility to Gram-negative mastitic bacteria was observed for the tested drugs, with *Enterobacter spp* and *Citrobacter spp* indicating a 100% level of susceptibility to all the tested antimicrobials; *Enterobacter spp* and *Citrobacter spp* showed 0% multi-drug resistance. However, *Staphylococcus aureus, Streptococcus spp,* and *Escherichia coli* indicated some levels of multi-drug resistance to the tested drugs.
- Finally, the study established that, although the education levels of camel keepers may determine the early diagnosis of sub-clinical mastitis, the main determinant factor is their knowledge of the appropriate mastitis testing method.

5.6 Recommendations

- Hygienic camel milk production should be emphasized at the farm level by always washing the camel udder before and after milking. Appropriate milking techniques should always be applied to prevent the spread of mastitis.
- Farmers should be trained on the appropriate mastitis screening tools and encouraged to carry out periodic screening and diagnosis on their livestock. To promote animal health, animal welfare training should be done for camel keepers.
- Sensitization on the importance of involving trained veterinary professionals in treating mastitis should be carried out by the government and stakeholder groups. This measure not only allows the use of effective drugs in the treatment of the disease but also prevents the risk of the development of antimicrobial resistance.
- For the advancement in the management of livestock health, new antimicrobials should be used to avoid overuse of the current ones.

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APPENDICES

APPENDIX 1: CHARACTERISTICS USED FOR IDENTIFICATION OF THE ISOLATED BACTERIA

APPENDIX 2: QUESTIONAIRE USED TO COLLECT DATA FROM INTERVIEWEES

UNIVERSITY OF NAIROBI

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCE

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY

SUBJECT: QUESTIONNAIRE

Date of interview .....……. Mobil No. ..... Code……

QUESTIONNAIRE ON PREVALENCE OF MASTITIS AND FACTORS ASSOCIATED WITH ITS OCCURRENCE IN DROMEDARY CAMELS IN BENAADIR REGION, SOMALIA

PART A: GENERAL INFORMATION

1. Questionnaire number:……

2. Farm code:…….....

3. Province:…….

4. District…….

5. Sector…….

6. Cell…….

7. Village…….

PART B: RESPONDENT'S PARTICULARS

1. Name of respondent ……

2. Gender: (a) Male ☐ (b) Female ☐

3. Age (years)

a) 15 – 20 yrs. ☐

b) 21 – 30 yrs. ☐

c) 31-40 yrs. ☐

d) 41-50 yrs. ☐

e) More than 50 yrs. ☐

f) Don't know or prefer not to say ☐

4. Which level of education have you attained?

a) Non-formal education ☐

b) Primary level ☐

☐

c) Secondary level

d) Tertiary level ☐

PART C: FARMING PRACTICES AND ANIMAL HEALTH MANAGEMENT

1. How many camels do you keep? ☐

A)15-25

B)30-50

C) More then

2. What farming system are you practising?

a) Extensive (never kept indoors)

b) Semi-intensive (kept outdoors during the day and kept indoors overnigh ☐

c) Intensive (primarily kept indoors – zero grazing) ☐

3. How many lactating camels do you have in this herd? ☐……

4. What is the water source for your animals?

a) Tap water ☐

b) Water pans/flood water ☐

c) Local River/streams ☐

d) Local wells/boreholes ☐

5. Do you know Mastitis?

a) Yes ☐ b) No ☐

6. At what time do you observe mastitis in your herd?

a) The first 3 months of lactation ☐

b) During the whole lactating period ☐

c) During the dry period ☐

7. How do you treat mastitis?

a) Seek for veterinarians ☐

b) Treatment by myself ☐

c) If yourself, what type of drug do you use?

- Antibiotics: Pen streptomycin ☐ Multiject ☐ Other (Specify) ☐

- Anti-inflammatory drugs: Phenylject ☐

- Antibiotics and anti-inflammatory drugs ☐

8. How do you prevent mastitis in your herd?

a) Use of teat dips ☐

b) Wash the udder and use udder towels ☐

c) Early treatment of new cases ☐

d) Apply dry therapy ☐

PART D: MILKING PRACTICES

1. How many times do you milk per day?

a) Once: in the morning ☐ In the evening ☐

b) Twice ☐

2. Which technique do you use?

a) Stripping (Pulling the teat) ☐

b) Squeezing Action ☐

3. Do you test for mastitis? a) Yes ☐ b) No ☐

4. If yes, which method do you use?

a) Strip cup ☐

b) California mastitis test ☐

c) Mastitis test paper ☐

5. What do you do to ensure clean milk production? ☐

a) Observe strict cleanliness ☐

b) Strain milk ☐

c) Milk healthy animals only ☐

d) Use healthy and clean personnel for milking ☐

7. Who primarily milks the lactating female camel? (multiple choice)

a) Myself ☐

b) Family member only ☐

c) External employees ☐

d) Others (specify)……

8. What do you do with milk from your animals?

a) For family consumption ☐

b) Sale to milk vendors/traders ☐

c) Sale to milk collection centres ☐

d) Sale to neighbours and members of the community ☐

9. How often do you wash your milk containers?

a) Before milking ☐

b) Just after milking ☐

c) Both ☐

10. What source of water do you use to clean milk handling equipment?

a) Tapped/piped water ☐

b) Wells ☐

c) Boreholes ☐

d) Water stream ☐

13. What type of water do you use to clean milk equipment?

a) Warm water ☐

b) Cold water ☐

c) Warm water and disinfectant (Specify the disinfect…….) ☐

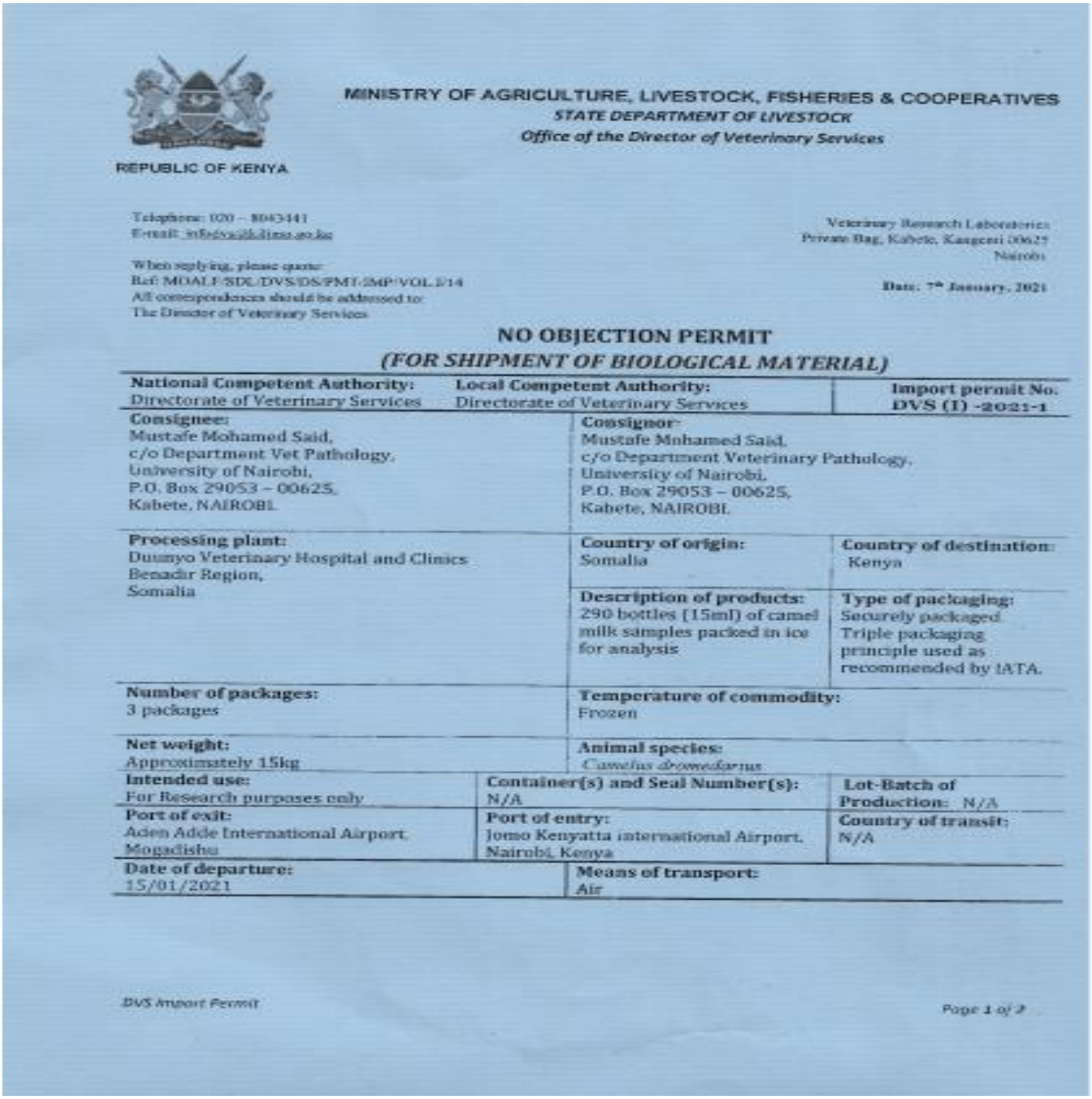
d) Cold water with disinfectant (Specify the disinf ……..) ☐

APPENDIX 3: SUSCEPTIBILITY PATTERNS OF THE 5 MOST-ISOLATED BACTERIA FROM THE STUDY MILK SAMPLES

Antibiotics	Classification of isolates: Number of isolates (%)														
	Staphylococcus aureus (n=26)			*Streptococcus* spp. (n=21)			*Enterobacter* spp (n=3)			*Citrobacter* spp (n=2)			*Escherichia coli* (n=4)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Gentamycin(GEN)	100%	0%	0%	*b*	*b*	*b*	100%	0%	0%	100%	0%	0%	100%	0%	0%
Ampicillin (AX)	57.69%	0%	42.31%	66.67%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%
Kanamycin(K)	100%	0%	0%	*b*	*b*	*b*	100%	0%	0%	100%	0%	0%	100%	0%	0%
Penicillin (P)	65.38%	0%	34.62%	0%	0%	100%	100%	0%	0%	100%	0%	0%	0%	0%	100%
Tetracycline(TE)	100%	0%	0%	66.67%	33.33%	0%	100%	0%	0%	100%	0%	0%	50%	50%	0%
Norfloxacin(NIX)	100%	0%	0%	*b*	*b*	*b*	100%	0%	0%	100%	0%	0%	100%	0%	0%
Erythromycin (E)	88.46%	3.85%	7.69%	76.19%	23.81%	0%	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
Streptomycin(S)	57.69%	0%	42.31%	*b*	*b*	*b*	100%	0%	0%	100%	0%	0%	100%	0%	0%

b: Breakpoints are not provided in the CLSI guidelines (2016); S: Susceptible; I: Intermediate and R: Resistance.

APPENDIX 4: NO OBJECTION PERMIT (FOR SHIPMENT OF BIOLOGICAL MATERIAL)



MINISTRY OF AGRICULTURE, LIVESTOCK, FISHERIES & COOPERATIVES
STATE DEPARTMENT OF LIVESTOCK
Office of the Director of Veterinary Services

REPUBLIC OF KENYA

Telephone: 020 – 8043441
E-mail: info@vsdlivestock.go.ke

When replying, please quote:
Ref: MOALF/SDL/DVS/DS/PMT/IMP/VOL.I/14
All correspondences should be addressed to:
The Director of Veterinary Services

Veterinary Research Laboratories
Private Bag, Kabete, Kangemi 00625
Nairobi

Date: 7th January, 2021

NO OBJECTION PERMIT
(FOR SHIPMENT OF BIOLOGICAL MATERIAL)

National Competent Authority: Directorate of Veterinary Services	**Local Competent Authority:** Directorate of Veterinary Services	**Import permit No.** DVS (I) -2021-1
Consignee: Mustafe Mohamed Said, c/o Department Vet Pathology, University of Nairobi, P.O. Box 29053 – 00625, Kabete, NAIROBI.	**Consignor:** Mustafe Mohamed Said, c/o Department Veterinary Pathology, University of Nairobi, P.O. Box 29053 – 00625, Kabete, NAIROBI.	
Processing plant: Duunyo Veterinary Hospital and Clinics Benadir Region, Somalia	**Country of origin:** Somalia	**Country of destination:** Kenya
	Description of products: 290 bottles [15ml] of camel milk samples packed in ice for analysis	**Type of packaging:** Securely packaged Triple packaging principle used as recommended by IATA.
Number of packages: 3 packages	**Temperature of commodity:** Frozen	
Net weight: Approximately 15kg	**Animal species:** *Camelus dromedarius*	
Intended use: For Research purposes only	**Container(s) and Seal Number(s):** N/A	**Lot-Batch of Production:** N/A
Port of exit: Aden Adde International Airport, Mogadishu	**Port of entry:** Jomo Kenyatta international Airport, Nairobi, Kenya	**Country of transit:** N/A
Date of departure: 15/01/2021	**Means of transport:** Air	

DVS Import Permit

Page 1 of 2

Conditions: -

1. The Consignment must be accompanied by a Veterinary Certificate endorsed by the Competent Authority of the country of origin. The certificate should quote the number of this import permit and indicate the type and number of specimens, origin, tests required, consignor, consignee, airway bill number/container seal and batch of production number as applicable.
2. The specimen for diagnosis or research purposes should only be handled in a laboratory licenced to handle infectious agents of risk group 2, 3 or 4. After completion of use, the specimens or animals used in in-vivo tests will be disposed appropriately/ by incineration.
3. Any other specimens not handled in a laboratory should be free from any **infectious agents** and **prions** and will be used for purposes stated only.
4. An inspection on arrival is required to verify that the imported goods are as listed in the permit This permit can only be used for the importation of specimens listed and cannot be used for the importation of any material
5. Misuse of this import permit for the importation of any other material will lead to the immediate cancellation of the permit, without prior notice being given.
6. The import permit is valid for two months and is subject to amendment or cancellation by the Director Veterinary Services at any time, without prior notice being given
7. Biological materials imported into this country must be packaged to withstand breakage and leakage of contents and be packaged and labelled, in accordance with the International Air Transport Association's (IATA) Dangerous Goods Regulations.
8. The materials cannot be transferred to another laboratory or person not listed in the permit
9. Persons who will work with the pathogens should ensure limited contact with species susceptible to the pathogens being used.
10. Specific conditions relating to the appropriate containment level and biosecurity procedures and practices should apply.

Date:
7th January 2021

Official stamp:

DIRECTOR OF VETERINARY SERVICES
KABETE VET LABS, KENYA

Signature:

Name: Dr. Romona Ndanyi

***For:* DIRECTOR OF VETERINARY SERVICES**