2022-07

# Sero-Prevalence of Camel Brucellosis and Its Public Health Importance in Selected Site of Salahley District, Western Somaliland

Abdikariim Ahmed


# BAHIR DAR UNIVERSITY

# COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

# SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

## GRADUATE PROGRAM

# SERO-PREVALENCE OF CAMEL BRUCELLOSIS AND ITS PUBLIC HEALTH IMPORTANCE IN SELECTED SITE OF SALAHLEY DISTRICT, WESTERN SOMALILAND

M.Sc THESIS

By

ABDIKARIIM AHMED YOUSUF

**July 2022**

**Bahir Dar, Ethiopia**


# BAHIR DAR UNIVERSITY

# COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

# SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

## GRADUATE PROGRAM

## SERO-PREVALENCE OF CAMEL BRUCELLOSIS AND ITS PUBLIC HEALTH IMPORTANCE IN SELECTED SITE OF SALAHLEY DISTRICT, WESTERN SOMALILAND

**M.Sc. THESIS**

**By**

**ABDIKARIIM AHMED YOUSUF**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MSc.) IN "VETERINARY PUBLIC HEALTH"**

**July 2022**

**Bahir Dar, Ethiopia**

## THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (MSc.) thesis open defense examination, we have read and evaluated this thesis prepared by Mr Abdikarim Ahmed Yousuf **"Seroprevalence of camel brucellosis and its public health importance in selected site of salahley district, western Somaliland"** We hereby certifies that, the thesis is accepted for fulfilling the requirements for the award of the Degree of Master of Sciences (MSc.) in **Veterinary Public Health.**

**Name of external examiner:**

Name ----------Signature ---------- Date ----------

**Name of internal examiner:**

Name: Dr.Yeshwas Ferede Signature ---------- Date -------25th August 2022

**Name of chairperson:**

Name----------Signature ---------- Date ----------

## DECLARATION

This is to certify that this thesis entitled **"Sero-prevalence of camel brucellosis and its public health importance, in Salahley district, western Somaliland"** submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in "**Veterinary Public Health**" to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Mr Abdikariim Ahmed Yesuf (ID. No. BDU 1209594) is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

Name of the student

Abdikariim Ahmed Yousuf

Signature_____ date _____

Name of the advisor

Mussie Hailemelekot (Dr)

Associate professor.

Signature_____ date_____

## ACKNOWLEDGEMENTS

I am thankful to my academic Advisors Dr. Mussie Hailemelekot for the, unreserved technical and professional advice, rectification of the research work, the critical review and editing and provision of valuable reference materials for the accomplishment of this manuscript. I would like to thank Bahir Dar University, School of post graduate studies for the overall coordination and support of this study. Furthermore, I am indebted to the director of Somaliland veterinary diagnostic and research laboratories, Dr. Yasir, for his kind cooperation and offering me to use freely materials needed for blood sample collection, reagents, equipment and facilities required for RBPT. Additionally, the Somaliland veterinary diagnostic and research laboratories staffs and others are thankfully acknowledged for their kind collaboration and provision of Laboratory and field facilities. Similarly, the camel owners' during data collection and examination of animals is thankfully self-proclaimed. The diligent field assistance of Mr. Abdilahi Idiris, Mr. Khalid Aden and Mr. Muhyadin Abdillahi who facilitated the cooperation of camel owners in Salahaly, Toon and Kabada districts, respectively, are gratefully acknowledged. Also the support of Golis University, College of Veterinary Medicine personals, namely Dr. Abdirahman Mohamed, Dr. Hassan Abdi, and others safeguarded the achievement of the field study. Besides Mr. Xamse deserves special credit due to his kind endowment of lodging in Toon district during my sample collection. Moreover, I would like to concede my gorgeous Father Ahmed Yousuf Hayd for his vow of assisting in any aspects during my research work. Lastly my gratitude will go to all members of my family, for their encouragement and true sweat heart that they always deserve for me.

## DEDICATION

This paper is dedicated to my mother, Nimco War same and my father Ahmed Yousuf, who never went to school themselves for raising me up and sending me to school and supporting me financially and morally throughout my academic career. To my beloved Aunt, Cibado Yusuf, and my uncle Ismaciil yousuf.

## LIST OF ABRIVATIONS

| | |
|---|---|
| AFAC | Abortion in female adult camel |
| CFT | Complement fixation test |
| ER | Endoplasmic reticulum |
| ICWR | Interaction of camel with ruminants |
| IDF | International Dairy Federation |
| IG | Immunoglobulin |
| ISABS | International Standard for Anti-Brucella abortus |
| LPS | Lipopolysaccharide |
| MDPH | Massachusetts Department of Public Health |
| MHCII | Major histocompatibility complex II |
| MRT | Milk ring Test |
| NK | Natural killer |
| OR | Odd ratio |
| OIE | Office International Des Epizootics |
| PAHO | Pan American Health Organization |
| RBPT | Rose Bengal plate test |
| SAT | Serum Agglutination Test |
| SNVL | Somaliland national veterinary lab |
| SRBC | Sheep Red Blood Cells |
| WHO | World Health Organization |

# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

Sero-prevalance of camel brucellosis and its public health importance in selected site of Salahley district, western Somaliland

Abdikariim Ahmed[1], Mussie Hailemelekot[2]

University of Golis, faculty of veterinary Medicine, 2. Bahir Dar University, School of animal Science & Veterinary Medicine

## ABSTRACT

Brucellosis is an infectious disease in domestic and wild animals with serious zoonotic and economic implication, being more severe in developing countries. Cross sectional study was done in Salahley district of western Somaliland with the objective of estimating seroprevalence, determine the potential risk factors of camel Brucella and estimate its public health impact. Three areas were selected on the basis of their camel population. Three hundred eighty-four camels and sixty camel owners were included for the study. Serum sample was taken from the jugular vein camel and arm vein of human and the sera were tested using RBPT as screening test and CFT as confirmatory test. The data were analyzed using STATA software 13 and association of risk factors was done using unavailable and multivariable logistic regression analysis. The overall individual and herd level significant of sero-prevalence of camel brucellosis in the study area were 4.6% and 6.7%, respectively. The seroprevalence with respect to district level was 2.8%, 5.5% and 5.9% in Salahlay, Toon and Kabada district, respectively. Multivariable logistic regression analysis indicated that age, herd size, previous history of abortion and camels that are kept closely together with other ruminants to be statistically significant risk factors for seropositivity of Brucella in camels with adjusted odds ratio (OR) of 3.14, 4.37, 6 .86 and 11.8, respectively. The overall seroprevalence of human brucellosis in the study areas was 3.3%. Moreover, the questionnaire survey revealed that most respondents in the study area (97%) did not know about zoonotic importance of Brucellosis and drink raw camel milk. Similarly, 63% of respondents mentioned that they touch aborted fetus and placenta bare hand. The current study disclosed that camel brucellosis is prevalent in the area; hence, the following recommendations are forwarded. Further epidemiological studies involving the role of other ruminants for the occurrence of camel brucellosis and transmission of the disease in pastoral areas is important, Awareness creation through public health educational programmes on modern animal husbandry and management systems of animal diseases and risk of zoonotic diseases including brucella is highly recommended

**Keywords**: Brucellosis, Camels, public health importance, Risk factors, Somaliland,

# 1. INTRODUCTION

## 1.1 Background and justification

Camel (*Camelus dromedarius*) belong to the family of Camelidae and have an effective socio-economic role in different parts of the world with dry and semi dry climatic condition (Alamian and Dadar, 2019). The major roles of camel are associated directly to its impressive adaptation to extremely harsh situations due to several anatomical and physiological characteristics (Ramet, 2001). Camels ensure food security in pastoral communities by producing milk and meat. They are also sources of hides, which are used as bed sheets; serve as means of transportation and draught power (Ahmad *et al.,* 2010). Long lactation and the ability to maintain milk production over long dry spells are important facets of camel production. In spite of all these advantages, camel production and productivity are constrained by a number of factors including infectious diseases, of which brucellosis is considered to play a major role (Köhler-Rollefson *et al*., 2001).

Camel brucellosis is a wide spread disease in camel rearing regions of the world such as middle East and the Arabian Gulf, parts of Africa, and Latin America with the exception of Australia (Bamaiyi *et al.,* 2014). The occurrence of camel brucellosis in sub-Saharan Africa (either prevalence or incidence) is not well documented and reports submitted to the World Organization for Animal Health (OIE) are largely confined to serological surveys, which are mainly conducted for cattle, sheep, goats and less for camel (Racloz *et al*., 2013). Persistent case of brucellosis was observed in most African countries like Tanzania, Nigeria, Uganda, Kenya, Zimbabwe and Somalia reporting brucellosis in humans and domestic animals such as: cattle, camels, goats and sheep (Racloz *et al.,* 2013).

In East Africa, brucellosis is reported in most member countries of IGAD and endemic with high economic loss and zoonoses (Zewdie Teka *et al* 2018). Camel brucellosis is a contagious disease usually caused by *Brucella abortus* and *B. melitensis*, less frequently by *B. suis*, all of which are Gram-negative, facultative, intracellular *coccobacilli* bacteria (Tadele Tolosa, 2004). *Brucella abortus* and *B. melitensis* are mainly infective for camel, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected (Radostits *et al*., 2007).

In 1897, Bernhard Bang, a Danish veterinarian and physician, identified *B. abortus* and *B. melitensis* the cause of abortions in camel. At the beginning of the century, Zammit identified goats as the reservoir of brucellosis in Malta. The relationship between the agents of Malta fever

and Bang's disease was recognized by Alice Evans, who renamed the genus Brucella to honor Bruce (Mariana *et al.,* 2009).

Sources of infection for the transmission of the camel brucellosis are aborted fetuses, the fetal membranes and vaginal discharges and milk from infected animals (Radostits *et al,* 2007; PAHO/ WHO, 2001). The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn camels, all of which may contain a large number of the organisms and constitute a very important source of infection and Also drinking infected unpasteurized milk/raw dairy products can have transmitted with adisease (PAHO/WHO, 2001).

The same organism also causes undulant fever in human from drinking raw or un-pasteurized infected milk or milk products or from exposure of farmers, packing house workers, veterinarians and others to infected discharges or tissues (Bekel Megersa, *et al*.2011).

### 1.2 Statement of the problem

In Somaliland, brucellosis is considered to be one of the most serious disease problems facing livestock and veterinaryprofession (ministry of livestock Somaliland, 2013). The high prevalence is probably due to the fact that the country has not yet started control or eradication schemes. However, only a few African countries have ever carried out an extensive survey of the prevalence of brucellosis in animals or human. According to (Kebede Messay.*et al*, 2008) brucellosis is perhaps the most widespread and economically important disease in tropical and sub-tropical regions.

Lack of awareness about zoonotic diseases, keeping different species of animals together at several conditions, existing habit of raw milk consumption and close contact with animals can serve as means of brucella infection to human. Moreover, the mixing of the different species during migration, at watering or in night enclosures (resting), between camels and small ruminants is visible. In fact, African pastoralists believe that camel milk has medicinal values only when it is drunk in raw status without heat treatment (Mammeri *et al.*, 2014*).*

In the Somali pastoralists, it is not applicable at all to boil and drink camel milk instead they consume raw milk, raw liver and they did not use any protective material while handling parturient camels, removing placenta and/or other aborted materials since most of the people

have Poor knowledge about brucellosis. Most of the camel owners believe that camel milk possesses superior storage life, medicinal properties (against dropsy, jaundice, diabetes, glycaemia) and has an aphrodisiac effect. However, most of them don't have any knowledge about the transmission of brucellosis from consumption of raw milk. Hence the isolation of *B. abortus* and *B. melitensis* (Gessese, A. T., *et al* 2014) has certainly demonstrated the danger of camel milk to public health.

In spite of the existence of risk factors for camel brucellosis in camel population and exposure of pastoral people for zoonotic brucellosis, very few information exists on the epidemiology and public health importance of camel brucellosis in the pastoral area of Somaliland. Thus, there is a need for further study on the Seroprevalence of camel brucellosis and associated risk factors for zoonotic transmission and public health importance and to design and implement control measures aiming at preventing further spread of the disease both in animal and pastoral communities.

## 1.3 Objectives of the study

### *1.3.1 General objective*

To determine the seroprevalence and public health importance of camel brucellosis in the Salahlay district Hargeisa Zone, Somaliland

### *1.3.2 Specific objective*

- To estimate the seroprevalance of camel brucellosis in the camel population of the study area
- To assess the public health importance of the camel brucellosis in study area.
- To identify possible risk factors for the occurrence of camel brucellosis

### *1.3.3 Research questions*

1. What is the Sero-prevalence of camel brucellosis in camel population of the study area?
2. What is the public health importance of the camel brucellosis in study area?
3. What are the possible risk factors for the occurrence of camel brucellosis?

# 2. LITERATURE REVIEW

## 2. 1. Etiology

The genus Brucella is divided into ten classified species and subdivided into biovars. The disease in dromedary camels is caused by *Brucella abortus* and *Brucella melitensis* (Hadush Angesom *et al.*, 2013. *Brucella abortus* and *B. melitensis* are the causative organisms for camel brucellosis and at least nine biotypes have been recognized including a number of strain variants (Radostits *et al.*, 2007). *Brucella abortus* and *B. melitensis* are mainly infective for camel, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected (Shoukat, Shabu, *et al*. 2017). Cattle can be also become infected by *B. melitensis* when they share pasture or facilities with infected goats, or sheep. The infections in camel caused by heterologous species of Brucella are usually more transient than that caused by *B. abortus* (PAHO/WHO, 2001) (Table 2.1)

**Table 2.1 List** of currently characterized Brucella species, their typical animal hosts, as well as Their potential cause of human disease

| **Species** | **Biovars** | **Animal host** | **Human disease** | **First description** |
|---|---|---|---|---|
| Brucella (*B. abortus*) | 1–9 | Cattle, bison, buffalo, elk, yak, camel | Yes | Schmidt, 1901; Meyer and Shaw,1920 |
| *B. melitensis* | 1–3 | Sheep, goat, cow , camel | Yes | Hughes, 1893; Meyer and Shaw, 1920 |
| | 3 | Nile catfish; dog | | |
| *B. suis* | 1 | Horse | Yes (biovars 1–4) | Cook and Kingston, 1988 |

Camels are most frequently infected by various biovars of the two species *B. abortus* and *B. mellitensis*. *B. abortus biotype 3* and *B. mellitensis* which is most virulent species of the genus Brucella and has three biovars, with biovars 1 and 3 being those isolated most frequently in camel and small ruminants (Blasco and Molina, 2011). The distribution of biovars could be important in ascertaining the source of some infections (Garin-Bastuji *et al.*, 2006).

### *2.1.1 Characteristics of Brucella*

*Brucella* is very small (0.5–0.7 μm × 0.6–1.5 μm), faintly stained Gram-negative coccoid rods, with a microscopic appearance of 'fine sand', that lack endospores, capsules, or native plasmids. Brucella bacteria are Gram-negative *coccobacilli* that are non-motile and non-spore-forming (OIE, 2008). Primary culture of *Brucella* reveals punctate, non-pigmented, and non-haemolytic colonies. Colonies of smooth (S) *Brucella* strains are raised, convex, circular and translucent. After sub- cultivation or prolonged culture (>4 days), the colony morphology of *Brucella* may become less convex and opaquer with a dull, dry, yellowish-white granular appearance. These changes are caused by the dissociation of *Brucella* from smooth to rough forms. *Brucella* species stain weakly with safranin. Although *Brucella* is a strict aerobe, some strains require carbon dioxide, especially on primary isolation. *Brucella* is non-motile and generally oxidase-positive and urease positive (Liu, 2015).

## 2.2 Epidemiology

The risk factors that influence the initiation, spread, maintenance and/ or control of camel brucellosis are related to the animal population, management and to biology of the agent (Radostits *et al.,* 2007). Brucellosis has a worldwide distribution and affects both human and animals such as cattle, pigs, sheep, goats, camelids, dogs and, occasionally, horses (Wernery *et al*., 2014). Brucella infections have also been documented worldwide in a great variety of wild life species and, more recently, in marine mammals (Godfroid, Jacques, *et al.* 2013).

The prevalence of the disease is usually high in open camel herd than closed herd because of the frequent transmission and spread from other animals (El-Amier *el at.,* 2017). The incidence appears to be closely related to breeding and husbandry practices. High animal and herd prevalence have been reported from different countries, which not only pose a severe risk to humans but also to other livestock. A sero-prevalences of dromedary brucellosis of 40% has been reported from Sudan, and the United Arab Emirates (UAE) has experienced a drastic increase of brucellosis in camel populations due to the uncontrolled import of dromedaries from East African countries including Ethiopia (Omer *et al*., 2010).

### *2.2.1 Resistance and survival properties*

The organism is reasonably resistant to environmental influences and under suitable conditions can survive for a long period in the environment. In conditions of high humidity, low temperature and no sunlight Brucella bacteria can remain viable for several months in water,

aborted fetuses, manure, wool, hay, equipment and cloths (Alegbeleye Oluwadara, 2018). Brucella are generally susceptible to heat, direct sun light, acidic conditions and common disinfectant (Danner *et al.,* 2006). However, in favorable conditions the organisms may survive four to six days in urine, six weeks in dust and four to ten weeks in water, 40 to 75 days in aborted fetus (Percin *et al.,* 2013). They also survive the production process of soft cheese up to six months, in butter up to four months, in milk up to six months and ice cream up to 30 days (Sprague *et al*., 2012).

Disinfectants reported to destroy Brucella on contaminated surfaces include 2.5% sodium hypochlorite, 2-3% caustic soda and 2% formaldehyde solution. Presence of organic matter and low temperature decrease the efficacy of disinfectants (Sprague *et al*., 2012).

### *2.2.2 Host and pathogen risk factors*

Susceptibility of camels to Brucella infection is influenced by the age, sex and reproductive status of individual animals (Mohamud Ahmed, *et al* 2022). Sexually mature pregnant she camels are more susceptible to infection with the organism than sexually immature camels of either sex. It can be a continuing problem in large flocks because of massive environmental contamination of areas used for pregnant and calving she-camel (Corbel, 2006).

In some areas the prevalence of camel brucellosis associated with *B. melitensis* is linked to the practice of animal movement to summer and mountain pastures where there is commingling of sheep and goats from a variety of sources on the same pasture (Parvizi *et al.,* 2009).

Numerous risk factors have been determined for human camel-derived brucellosis including consumption of unpasteurized camel milk and buttermilk, unpasteurized dairy products, close contact with animals, camel ownership assistance during animal parturition and the presence of further infected family members (Sprague, *et al*, 2012). A number of studies have reported that the highest prevalence can be found in males; however, studies from Saudi Arabia, Oman, and Jordan have shown that contrary to common belief, children can also be strongly affected by brucellosis with prevalence between 21 and 70% (Al-Majali *et al* 2009).

### *2.2.3 Management risk factors*

In general, brucellosis can be found in any season of a year, the epidemic peak occurs from February to July and is closely related to the months associated with delivery and abortion in animals (Gul and Khan *et al.* (2007). In humans, prevalence of the disease is high (39.5%) in

summer season. Camel brucellosis caused by *B. abortus* or *B. melitensis* biovars can be encountered in all camel rearing countries with the exception of Australia (Daneshvar *et al*., 2003). High individual animal and herd prevalence have been reported from numerous countries, which not only pose a continuous risk for human infection, but also increase the spread of infection through uncontrolled trade of clinically inconspicuous animals. Several risk factors have been identified for camel brucellosis, these are at animal level (Al-Majali *et al.*, 2009).

Further risk factors are the increase in species composition at household level, and the wet season. Cattle, swine, goats, and sheep are the most common reservoirs of Brucella spp (Alshwany, 2019). Bison, elk, caribou, and some species of deer may also harbor Brucella spp. Camels appear to become infected via spill-over from small ruminants and cattle (Sprague *et al*., 2012).

This observation is supported by the fact that all Brucella spp. and biovars infecting other ruminants have also been isolated from camel. Recent reports from different countries indicate that there is an epidemiological association between bovine, caprine, ovine and camel brucellosis. In sheep and goats herded with cattle and camels the prevalence of the disease were higher than those herded separately (Isam *et al*., 2016).

### 2.3 Mode of transmission and route of infection

The source of infection is the infected carrier ruminants; excretion is from the reproductive tract and in milk. Reproductive tract of infected does and ewes, whether they abort or birth normally, discharge large numbers of Brucellae in their uterine exudates and placenta (Megid *et al,* 2010). The organism can be present in uterine discharge for at least two months following parturition in infected goats the vaginal exudate of infected virgin or open animals may also contain the bacteria (Larsson *et al.*, 2005). Animals infected during pregnancy will excrete the organism in milk in the subsequent lactation and many will excrete it in all future lactations. In sheep the period of excretion of the organism from the uterus and in milk is usually less than in goats but the organism can be present in milk throughout lactation The duration of excretion in cattle is not known (Greenfield *et al*., 2002).

Since camels are not known to be primary hosts of Brucella, the transmission of camel brucellosis depends on the Brucella species being prevalent in other animals sharing their habitat and on husbandry (Gwida *et al* 2012). Among animals, the predominant route of exposure for smooth strains of Brucella is through ingestion or inhalation of organisms that are present in

fetal fluids or other birth products; herds are typically exposed following the introduction of an infected animal that subsequently gives birth or aborts a fetus, whereupon pasture or water become contaminated by these excretions (Hadush Angesom *at el* 2013). Transient disease (e.g., abortions) can also develop following administration of a live Brucella vaccine, particularly the *B. abortus* vaccine strain 19 (Waring, 2005).

The incubation period for Brucellosis is highly variable ranging from 5–60 days; illness most commonly occurs about one month after exposure. The disease normally does not spread from person to person, but in a few cases, women have passed the disease to their infants during birth or through their breast milk. Rarely, brucellosis may spread through sexual activity or through contaminated blood or bone marrow transfusions (Musa *et al.,* 2008). Major virulence factors are: lipopolysaccharide (LPS), T4SS secretion system and BvrR/BvrS system, which allow interaction with host cell surface, formation of an early, late BCV (*Brucella* Containing Vacuole) and interaction with endoplasmic reticulum (ER) when the bacteria multiply (Głowacka, Patrycja, *et al,* 2018).

### 2.4 Pathogenesis

Brucella spp. can enter the body through the lungs, the digestive tract, mucous membranes, and intact skin after penetration, the organisms are phagocytized by neutrophils and macrophages which carry them to the regional lymph nodes where they multiply and induce a lymphadenitis which may persist for months once in the blood stream, the organism disseminates to multiple organs, there by displaying an affinity for reticulo-endothelial tissues, such as liver, spleen, the skeletal, hematopoietic system and both male and female reproductive tracts, where it causes localized infection (Greenfield *et al*., 2002).

The ability of Brucella to replicate and persist in host cells is directly associated with its capacity to cause persistent disease and to circumvent innate and adaptive immunity the organism is able to escape phagocytic killing through inhibiting the phagosome-lysosome fusion and reproducing inside macrophages. Persistent infection is a common feature of the disease with frequent shedding of the bacterium in body secretions (Tanko *et al*., 2013). These bacteria have a predilection for the pregnant uterus, udder, testicles, accessory male sex glands, lymph nodes, joint capsules and bursa; lesions may be found in these tissues (Wernery, 2014).

## 2.5 Immunity

### *2.5.1 Humoral immune response*

Naturally infected animals and those vaccinated as adults with strain 19 remain positive to the serum and other agglutination tests for long periods (Poester *et al*., 2010). The serum of infected camel contains high levels of IgG1, IgG2, IgM, and IgA isotypes of antibody (Radostits *et al*., 2007). Similar isotypes at different relative concentrations occur in milk, although most of the IgA is present in secretory form (Yohannes Mollalegn, *et al.*, 2012). The first isotype produced after an initial heavy infection or strain 19 immunization is IgM and is soon followed by IgG1 immunoglobulin most abundant in serum and exceeds the concentration of IgG2 (Simister, 2008).

The magnitude and duration of the antibody response following immunization is directly related to the age at immunization and the number of organisms administered Following immunization with a standard dose of strain 19, IgG antibody concentrations usually decline to diagnostically insignificant levels over 3-6 months (Godfroid *et al.,* 2010).

### *2.5.2 Cellular immune response*

Brucella species are facultative intracellular pathogens (Godfroid *et al.*, 2010). They are readily phagocytized by macrophages and polymorphonuclear leukocytes and, in the case of virulent strains, are capable of surviving within these cells, and phagocytosis is promoted by antibody (Emmerzaal *et al.,* 2002). However, since virulent Brucella can survive within normal macrophages for long periods, recovery from infection is likely to be dependent upon the acquisition of increased bactericidal activity by phagocytic cells (Poester *et al.*, 2010). The release of these activating factors is dependent upon recognition of the appropriate antigen by the T- lymphocyte and is subject to regulation through the major histocompatibility complex (MHC II) (Simister, 2008).

Live organisms capable of establishing persistent intracellular infection and certain types of antigen, with or without adjuvant, are the most effective inducers of cell-mediated immunity (Banai, 2010). The role of cytotoxic cells, including cytotoxic T-lymphocytes, natural killer (NK), and killer (K) cells in the cell mediated immune response to Brucella has not been elucidated (Pulendran *et al*., 2010).

## 2.6 Clinical features

Brucellosis is characterized by abortion which usually occurs only once and to a lesser extent by orchitis and infection of the accessory sex glands in males (Wernery, 2014). According to various researchers, the clinical signs of brucellosis in breeding camelids are the same as those in bovines and small ruminants, although infection in breeding camelids causes fewer abortions than it does in bovines and small ruminants, some authorities feel that the most significant result of infection may be premature birth (Wernery, 2014). Infections may cause stillborn calves, retained placenta, fetal death and mummification and reduced milk yield. Also, delayed service age and fertility have been reported (Musa, 2001). A retained placenta is rare in Camelidae this may be a result of the difference in the placental attachment as they possess a diffused type of placenta like that of horses not cotyledonary placenta (Fowler, 2010).

According to the study done by (Damir *et al* (2021) non-pregnant dromedaries (n= 6) were artificially infected subcutaneously in the right lower hind of the neck with two strains of *B. abortus* (four with S19, two with field bovine strain, × $10^6$ bacteria,) developed only mild clinical signs. Reduced appetite, slight lameness and bilateral lacrimation were observed. On necropsy the pathogen was re-isolated 45 to 65 days later from the cranial and genital lymph nodes (Damir *et al*., 2021).

No clinical signs were observed in the four camels inoculated with S19, whereas slight nonspecific signs were found in the dromedaries infected with the bovine *B. abortus* field strain on necropsy, no gross lesions were detected, but histological results revealed focal granulomas in the liver and a generalised lymphadenitis (supra-mammary lymph node) the pathogen was re isolated from the lymph nodes of the genital tract and head (Damir *et al*., 2021).

In human acute brucellosis may begin with mild flu like symptoms, or symptoms such as: abdominal pain, back pain, chills, excessive sweating, fatigue, intermittent fever, (so called "undulant" fever because the fever rises and falls in waves, Malta Fever, Mediterranean Fever and Rock fever ) where high fever spikes usually occur every afternoon, headache, joint pain, depression, anorexia, weakness, weight loss and generalized aching, localized and chronic infections of organs (including the liver and spleen) can occur (El-Radhi, 2018).

## 2.7 Diagnosis

Diagnosis and control of brucellosis in camels must be carried out on a herd basis. The identification of one or more infected animals is sufficient evidence that infection is present in

the herd, and that other serologically negative animals may be incubating the disease and present a risk (Corbel, 2006). Diagnostic tests can be applied with different goals: confirmatory diagnosis, screening or prevalence studies, certification, in countries where brucellosis is eradicated and or surveillance in order to avoid the reintroduction of brucellosis through importation of infected animals or animal products (Corbel, 2006). Control of brucellosis in livestock and humans depends on the reliability of the methods used for detection and identification of the causative agent (Gwida *et al.*, 2011).

However, diagnosis of brucellosis in camels is frequently difficult. The disease can mimic many infectious and non-infectious diseases characteristic clinical signs of brucellosis in camels are often lacking and diagnostic methods are not evaluated yet (Gwida *et al.,* 2011). It may be suspected based on clinical signs such as abortions, but confirmation is made through serological tests, then with prescribed laboratory tests to isolate and identify the bacteria, following the guidelines describing the methods and diagnostic thresholds in the OIE (Marquez Aurélie, *et al*, 2017).

### *2.7.1 Identification of the agent*

#### *2.7.1.1 Microscopic examination*

This is a useful procedure for examination of abortion materials. Smears of placental cotyledon, fetal stomach contents or uterine exudates should be heat fixed and stained by a Stamp's modification of the Zeihl-Neelsen stain Brucella is a small, Gram-negative *coccobacilli* or short rod measuring 0.6 to 1.5µm by 0.5 to 0.7µm (Radostits *et al*., 2007; Tolosa, T. 2004).

**Animal Inoculation:** Into Guinea pig and mouse is the technique that has value for the isolation of Brucella when specimens are derived from potentially contaminated sources such as milk, cheese, semen, or genital discharges (Alton *et al* 2010). Inoculation should be made subcutaneously into Guinea pig or intravenously (0.1ml), or subcutaneously if the material is heavily contaminated, into mice (Lopez-Goňi *et al*., 2011). The guinea pig is killed 3 weeks post infection and the second 6 weeks after inoculation. A blood sample for serological examination is taken at the time of killing; macroscopic lesions are recorded and the spleen is cultured. The mice are killed 7 days after inoculation and the spleen and liver removed for culture on nutrient medium.

### *2.7.2 Serological tests*

When bacteriological diagnosis is not practicable diagnosis has to be based on serological methods, e.g. in surveys or eradication programs.

#### *2.7.2.1 Herd surveillance test/ Milk ring test (MRT)*

It is the most practical and economical method for locating infected dairy herds and for surveillance of brucellosis free herds. If performed on pooled milk 3 or 4 times a year on each herd, it will detect the majority of infected herds. Herds with a positive milk-ring test can then be examined by individual serum or milk tests to identify the infected individuals. Milk from individual animals can be serially diluted in Brucella-free milk to determine the end titre of the milk-ring reaction. Titres above 1:10 are suggestive of infection (Smite, 2013).

#### *2.7.2.2 Rose Bengal Plate Test (RBPT)*

Rose Bengal Plate Test (RBT) it is one of the easiest methods to implement and the most widely used for identifying Brucellosis antibodies in sera. Principle of the test: The RBPT is a rapid slide agglutination test, it is now often used widely for diagnosing disease the test uses a suspension of *B. abortus* smooth cells stained with Rose Bengal dye (pink color) to detect Brucella agglutinins (Alton *et al*, 2010). The stained bacterial suspension agglutinates when mixed with samples containing specific IgG or IgM antibodies present in the patient sample. The test is rapid, inexpensive, have 100% of sensitivity and 98% of specificity. Limitation is Low sensitivity particularly in long chronic cases, and relatively low specificity in endemic areas & vaccinations may produce agglutinins capable of reacting with the febrile antigens (Alton *et al*, 2010).

#### *2.7.2.2 Complement Fixation Test (CFT)*

Complement Fixation Test (CFT) is used as confirmation test for brucellosis. It provides the detection of anti-Brucella antibodies that are able to activate complement but in camel sera for testing in the CFT should be inactivated at 54 °C or 56 °C for 30 minus to high specificity and high sensitivity than any other conventional tests, it has been recognized as a confirmatory serological test for brucellosis (Jay, Maryne, *et al*. 2018). This test is a "prescribed test for trade, since this test is difficult to standardize, it is progressively being replaced by primary enzyme-linked Immiunosorbent assays the ELISA has not been widely evaluated for camel species, but is potentially useful subject to adequate standardization (Poester *et al.*, 2010). The CFT sensitivity ranged from 88.9% to 98.7% and mean CFT specificity from 89.3% to 100%.

#### *7.2.2.3 Enzyme Linked Immuno Sorbent Assay (ELISA)*

Enzyme Linked Immunosorbent Assay are divided into two categories, the indirect ELISA (iELISAs) and the competitive ELISA (cELISAs). Most iELISAs use purified smooth LPS as antigen and detect mainly IgGs or IgG sub-classes, their main quality is their high sensitivity but they are also more vulnerable to non-specific reactions, notably those due to infection (Godfroid *et al*., 2010).

The ELISA tests offer an excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit, a comparison with the SAT, ELISA yields higher sensitivity and specificity. The test is rapid, inexpensive, have 100% of sensitivity and 98% of specificity. Enzyme Linked Immunosorbent Assay (ELISA is also reported to be the most sensitive test for the diagnosis of neuro brucellosis (Schwarz, Norbert Georg, *et al*, 2017). The protein is now being used in an indirect plate ELISA system and has been evaluated with good sensitivity and specificity on large number of clinical samples (Hotam *et al* 2011).

#### *2.7.2.4 Serum Agglutination Test (SAT)*

In serum agglutination tests (SAT) an antigen reacts with its corresponding antibody, resulting in visible clumping of bacterial cells. With latex agglutination tests, latex particles are coated with antibodies that agglutinate specific antigens and form a more easily visible precipitate. Sensitivity and specificity of serum agglutination test is 100% and 81% (Moraveji *et al.*, 2012).

### *2.7.3 Molecular test*

#### *2.7.3.1 Polymeraze chain reaction (PCR)*

Polymerase chain reaction (PCR) is an in vitro technique for the nucleic acid amplification, which is commonly used to diagnose infectious diseases. The use of PCR for pathogens detection, genotyping and quantification has some advantages, such as high sensitivity, high specificity, reproducibility and technical ease (Moraveji et al., 2012). The direct culture and immunohistochemistry can be used for detecting infection with Brucella spp. However, PCR has the potential to address limitations of these methods. Polymerase chain reaction are now one of the most useful assays for the diagnosis in human brucellosis (Wang, Ying, *et al.* 2014).

## 2.8 Significance of the disease

### *2.8.1 Economic importance*

On an average, outbreak of camel brucellosis resulted in a loss of milk production of the herd as much as a 20% and this can reach 40-50% in early abortion (Tadele Tolosa, 2004). In addition to the loss of milk production, there is the loss of camels and interference with the breeding programs. This is of greater importance in camel herds where young camel represents the sole source of income (Radostits *et al.,* 2007). The common sequel of infertility increases the period between lactations, and in an infected herd the average inter parturition period may be prolonged by several months (Hadush Angesom *et al,* 2013).

Losses in animal production due to the disease can be of major importance, primarily because of the decreased milk production by aborting camels and this is often associated with retained placenta, metritis and a subsequent period of infertility (Radostits *et al.,* 2007). In general, economic losses due to brucellosis are usually caused by, losses due to abortion, diminished milk production, cull and condemnation of animals due to breeding failure, endangering animals export trade of a nation. Human brucellosis causing loss of some hours and medical costs, government costs on research and eradication schemes. Each year half a million case of brucellosis occurs in humans around the world. The prevalence of infection in animal reservoir provides a key of its occurrence in humans (Scholz *et al.,* 2011). Sero-prevalence of camel brucellosis in some North and sub-Saharan African countries is indicated in (Table 2.2).

**Table 2.2. S**ero-prevalence of camel brucellosis in some North and sub-Saharan African countries

| District | No. of samples | Test employed | Prevalence% | Reference |
|---|---|---|---|---|
| Sudan | 2000 | RBPT and cELISA | 39.9 and 40.5 | (Omer and Musa, 2010) |
| Libya | 14 | RBPT/ and /SAT | 9.67/8.06 | (Musa *et al*., 2008) |
| Somalia | 1246 | RBPT and iELISA | 3.9 and 3.1 | (Kalimuddin, 2010) |
| Chad | 288 | RBPT | 0.4 | (Berhanu Tilahun *et al.,* 2013) |
| Ethiopia | 1073 | RBPT | 2.1 | (Bekele Megersa *et al.,* 2011) |

### *2.8.2 Public health importance of brucellosis*

Brucellosis is a disease of animals in which human is infected as terminal host. The incidence of brucellosis in human is clearly correlated to the degree of incidence in the domestic animals around him (Moreno, 2014). Brucellosis is an infectious, contagious, and worldwide spread form of an important zoonotic disease caused by bacteria of the genus Brucella. Brucellae are facultative intracellular parasites of the reticuloendothelial system. The disease primarily affects cattle, sheep, goats, swine, and dogs. Among the members of the Brucella group Brucella abortus, B. melitensis, and B. suis species are not host-specific, and may transmit to other animal species; hence, from epidemiological evidence, the three species (B. abortus, B. melitensis, and B. suis) have distinct host preferences and the organisms are capable to cause an infection in a wide range of host species, including humans. The remaining three members of the species have much greater host specificity. Cross transmission of brucellosis can occur between cattle, swine, sheep and goats and other species including dogs, horses, feral swine, bison, rein deer and camels (Than, 2007).

**Table 2.3** Sero-Prevalence of human brucellosis in some African countries

| Country | Number Tested | Prevalence % | Tests | Reference |
|---|---|---|---|---|
| Nigeria | 13999 | 7.6-29.8 | SAT | (Chukwu, 1985) |
| | 738 | 5.55 | SAT | |
| Tanzania | 540 | 22.6 | SAT | (Chukwu, 1985) |
| | 80 | 20 | SAT | |
| Uganda | 3164 | 6.4 | SAT | (Chukwu, 1985) |
| Somalia | 353 | 0.6 | SAT | (Hussien *et al.*, 1987) |
| Djibouti | 108 | 6.5 | CFT | (Chantal et al., 1996) |
| Eritrea | 130 | 7.1 | CFT | (Omer *et al.*, 2002) |
| Ethiopia | 250 | 7.6 | CFT | (Adugna et al 2021) |

## 2.9 Control and eradication

Control of camel brucellosis should suite the conditions of the particular country where camels are raised vaccination of uninfected animals is generally considered the most effective and economical means of protecting livestock against brucellosis (Gwida *et al.*, 2012).

Consequently, vaccination should be performed on all negative reactors immediately after the third serological testing, to avoid the possible presence of carrier animals (Radwan *et al.,* 2020).

In most of the developing pastoralists, countries prevalence of brucellosis is low thus control by herd immunization and vaccination of calves at 4 to 8 months of age is helpful using S19 or Rev 1 vaccinal strains preceded by blood testing using the SAT or card test on the field (Gwida *et al.,* 2012). Seropositive animals should be identified and subjected to retesting. Additionally, test and slaughter policy can be followed in countries where intensification is practiced (Abbas and Agab, 2002).

### *2.9.1 Chemoprophylaxis*

The treatment of brucellosis in the camel has generally been unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs, the bacteria are facultative intracellular which survive and multiply within the cells (Radostits *et al*., 2007). Generally, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Plummer, Paul J., *et al,* 2018).

### *2.9.2 Immunoprophylaxis*

Vaccination of exposed herds with inactivated or live vaccines, only camel vaccination performed on young female camel between ages of 4-10 months, Vaccinated camels must be identified by a tattoo and ear tag. Adult vaccination the whole herd is vaccinated whenever there are certain problem herds have to be maintained in quarantines until all vaccinated animals have been removed from the herd (Bekele Megersa 2012). The following are some of the vaccination available against brucellosis:

***Killed B .abortus and B. melitensis 45/20 vaccines***

Two doses administered 6 weeks apart in animals over 6 months of age are required with Br. abortus and *B. militensist* 45/20 Adult camel vaccination is sometimes performed as a regulatory effort to control infection in a herd ,*Brucella abortus* vaccines play a central role in bovine brucellosis control/eradication programs and have been successfully used worldwide for decades. Strain 19 and RB51 are the approved *B. abortus* vaccines strains most commonly used to protect cattle against infection and abortion. However, due to some drawbacks shown by

these vaccines much effort has been undertaken for the development of new vaccines, safer and more effective, that could also be used in other susceptible species of animals (Thakur *et al*., 2012).

### *2.9.3 Test and slaughter*

Test and slaughter, involves recognition of all animals which have responded immunologically to Brucella infection and subsequent culling of the reactors according to these method could be achieved when the rate of infection is reduced to an acceptable level (about 1- 2%). Part of the scheme has to be a careful control of all animals which will be newly added to the herd as well as a production system which prevents contact with infected neighbouring farms and/or contaminated feed or pasture (Tadele Tolosa, 2004).

### *2.9.4 Hygienic Prophylaxis*

From the epidemiology of the disease, important steps were derived at an early stage as hygienic prophylactic measures. These include: Aborted fetuses, placentas, and uterine discharges must be disposed of, preferably by incineration (Radostits *et al.,* 2007).

Chlorhexidine gluconate is an effective antiseptic against *B. abortus* and is recommended for washing the arms and hands of attendants and veterinarians who are exposed to contaminated tissues and materials (Radostits *et al.,* 2007). Replacement stock should be purchased from herd free of brucellosis Camels, which are in advanced pregnancy, should be kept in isolation until after parturition, since occasional infected camels may not show a positive serum reaction until after parturition or abortion (Radostits *et al*., 2007).

# 3. MATERIALS AND METHODS

## 3.1 Study area

The study area was Salahley district. Salahley is a district in Maroodi Jeex Region (Hargeisa city) of Somaliland. It is located south of Hargeisa, the capital of the country. During Somaliland's second administration (1993-2002), Salahlay was incorporated as a district of Salahley in the Maroodi Jeex Region. It has Latitude of 9.0260° N and longitude of 44.2058° E, the average annual temperature of Salahley is 21.4 °C. Salahlay district area have four distinct seasons that comprise the spring rains of April to June, a dry summer from July to September, the autumn rains of October and November and a dry winter from December to March. The main animals raised in this area are goats, sheep, camels and cattle. The main livestock production system in the area is pastoral production (Hamse, 2016).

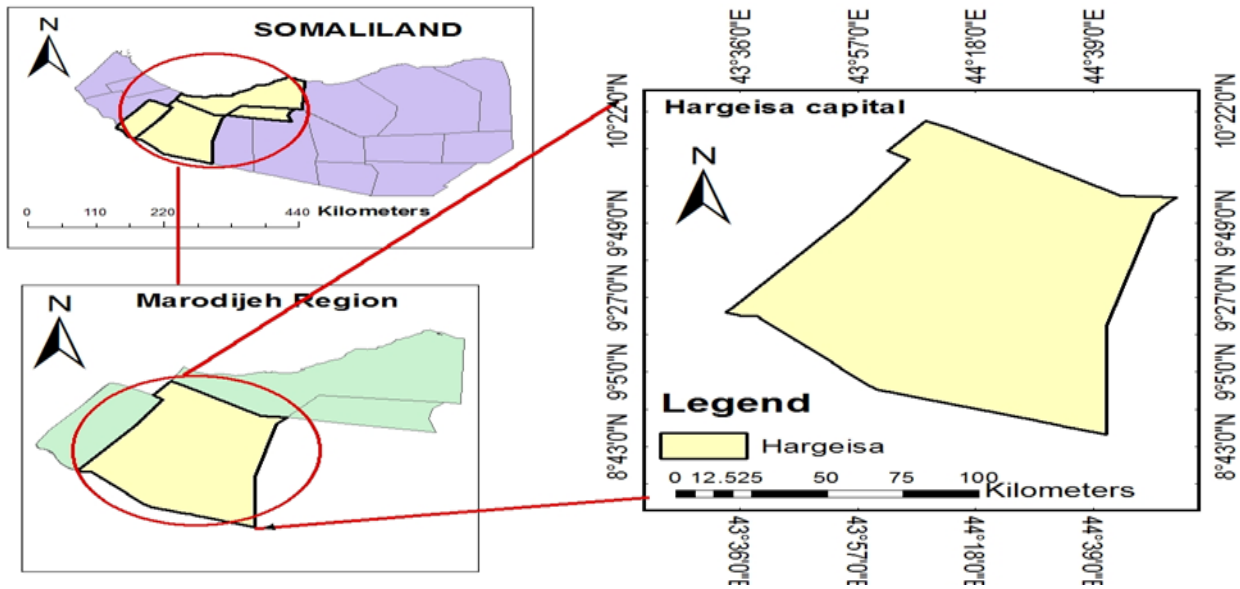


**Figure.1** Map of the study area (ministry of planning in Somaliland)

## 3.2 Study population

The target study population was local Salahley zone camels (*Camelus dromedaris)* managed under extensive pastoral production system by the pastoralist. The study camels were drawn from the three areas in Salahley district and these were Salahley, Toon and kaabada. The study camels were proportionally assigned among mentioned district areas. Camels aged 2 and above 2 years old were included in the sampling since camel under two years is young in age; therefore

is not target of the study Ahmadi (2005). The age category of camels was that above four years of age as matured (at age of puberty), while camels that are four and less than 4 years old were considered sexually immature, herd consisting 3-15, 16-25 and >25 camels was also considered as small, medium and large herds, respectively (Timiras, 2007). The camel population in each area are showing in (Table 3.1).

**Table 3.1.** .Camel population of the each selected districts of Salahley zone

| **Town** | **Camel population** |
|---|---|
| Salahlay | 9842 |
| Toon | 5466 |
| Kaabada | 7875 |
| **Total** | **23,183** |

## 3.4 Sample size and sampling method

Sample size was determined according to Thrusfield (2005) for random sampling and calculated using the expected prevalence of 50% (Berhanu Tilahun *et al*., 2013), 95% confidence interval and 5% absolute precision in the formula as follows:-

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

Where n = sample size, d = desired absolute precision (0.05) and Pexp = expected prevalence (50%). The minimum sample size calculated was 384. Proportional distribution of the sample was carried out depending on the camel population in the study areas.

## 3.5 Study design

A cross-sectional study was conducted on 384 one humped camels, in selected pastoral and agro-pastoral residences of the Salhaly, Kabada and Toon districts of Hargeisa, Somaliland, from October, 2020, until June, 2021, to estimate the sero-prevalence of Brucella infections with emphasis on potentially associated risk factors.

The three districts were purposively selected based on their accessibility and distribution of camel population in the areas. Among the districts, a total of 10 settlements or pastoral associations (5 villages from Salhlay, 3 villages from Toon and 2 villages from Kabada district) were purposively selected based on distribution of camel population.

Camels found in these settlements were the study population, where individual animals have been sampled using systematic random sampling. No camel was selected if a group contains less than three camels. Camels that were 2 years of age and above were sampled and included in this study. Moreover, 60 willingly selected camel owners (20 from Salhaly, 25 from Toon and 15 from Kabada, districts), living in the selected peasant associations, whose animals were tested for brucellosis have been included in the questionnaire survey.

## 3.6 Method of data collection

### *3.6.1 Questionnaire*

#### ***A. Camel***

A questionnaire was designed to collect information on factors that are believed to influence the spread and prevalence of Brucella infection. These include herd size and composition, management system, age of the animal, purchase source and replacement dairy camel, handling of animal product and handling of abortion, history of abortion and history of retained fetal membrane, this questionnaire were involved 60 camel owners.

#### ***B. Human***

With regard to the public health significance of the disease, the presence of symptoms suggestive of brucellosis in humans (fever, sweat, anorexia, malaise, weight loss, depression, headache and joint pains), the habit of consumption of un-pasteurized milk, contact with aborted animals or aborted materials and handling of parturient animals was considered and was administered to selected 60 individual camel owners to assess public health importance of brucellosis in human.

### *3.6.2 Collecting and handling of blood*

Camel & Human

Approximately 10ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes, needles and needle holder, each sample was labeled by using codes describing the specific animal. Similarly blood sample was collected from 60 camel owners. The blood samples were left at room temperature overnight, to allow clotting, for sera separation centrifuged at 2000-3000 rpm for 10-15 minutes. Then, the sera were separated from the clotted

blood by decanting to other tubes and were stored at −20°C until serologically tested. Rose Bengal plate test (RBPT) and complement fixation test (CFT) were used serially for screening and confirmatory test, respectively.

*3. 6. 3 Rose Bengal Plate Test*

Rose Bengal plate Test (RBPT): The RBPT test was carried out according to the method recommended by OIE, (2004).The antigen used for RBPT was obtained from (Institut Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France). This test was carried out at ministry of livestock and Somaliland national veterinary laboratory. Antigen and sera required for each day for serological testing was taken out from the cold storage (in refrigerator at 240 $C^{0}$) and brought to room temperature for 30 minutes before testing takes place. The antigen is stored at 4 $^{0}$C but the sera at -20 0C

**Test Procedures:**

Sera (control and test sera) and antigen for use were left at room temperature for half an hour before testing, since active materials straight from the refrigerator react poorly

1. 30 µl serum was mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter.
2. The antigen and serum was mixed thoroughly using an applicator stick (a stick being used only once)
3. The plate was rocked by hand for about 4 minutes
4. The tests was read by examining for agglutination in a good light
5. Magnifying glass is used to detect micro agglutination when suspected the interpretation will be performed as follows: 0 = no agglutination + = barely perceptible ++ = fine agglutination, some clearing +++ = coarse clumping, definite clearing those samples identified with no agglutination was recorded as negative those with +, ++, +++, ++++ were recorded as positive. (Nielson, 2002).

*3.6.4 Complement Fixation Test (CFT)*

All sera which are tested positive by the RBPT were further retested, using the CFT, for confirmation and the CFT test was done at Somaliland national veterinary laboratory (SNVL). Standard *B. abortus* antigen for CFT (from the Veterinary Laboratories Agency, Addle stone,

United Kingdom), Amboceptor and sheep red blood cells (SRBCs), ministry of livestock, Somaliland, was used to detect the presence of brucella antibodies against brucella antigen in the sera. Similarly, the control sera and complement used in this test was also obtained from SNVL. As an interpretation the test serum having SRBCs sedimentation at a dilution of ≥1:5 at adilution of 1:8 or above was considered positive for the disease the CFT have high specificity and high sensitivity (Ahmadi 2005). Serial dilution is accepted if 1:8

**Test procedure**

1. The sera was pre diluted at 1:2.5 and incubated at 58 $^0$C in a water bath for 30 minutes in order to inactivate the native complement
2. 25 µl of diluted test sera was placed in wells of first and second rows of U-bottom plate, and 25 µl of veronal buffer are added to all wells except those of the first row
3. Serial doubling dilution is then made by transferring 25 µl volumes of serum from 2 nd row on wards continuing for at least four dilution
4. 25 µl of antigen diluted to working dilution excluding those of anticomplemetary controls, which received 25 µl VBD was added to all wells
5. 25 µl of complement (1: 40 working dilution) in working dilution are added to all wells except control wells.
6. Control wells containing: serum control has serum + complement + diluent + and antigen control has antigen + complement + diluent. Complement control has complement + diluent and hemolytic system has diluent set up to contain 75µl total volume in each case before hemolytic system was added
7. The plates were incubated for 30 minutes at 37 C$^0$ with agitations ( warm fixation)
8. 25 µl of 2 % SRBC and amboceptor (hemolytic system)mixture is added into all the wells

Plates are sealed with sealing tape and placed on a shaker) and incubator (37 C$^0$) for 30 minutes.

**The interpretation**

The interpretation was performed as follow: Sera with at least 75 % fixation at adilution of 1:5 or 8 and at least 50 % fixation of the complement at a dilution of 1: 10 or above was considered as positive (OIE, 2004).

### 3.8. Data analysis and interpretation

The generated data on serum sampled on individual animals and questionnaire was carefully stored and entered on to Microsoft Excel spreadsheet (Microsoft Corporation) as database. Data on serum sampled

individual animals that are entered on to Microsoft Excel spreadsheet was imported to STATA version 13.0 for windows (Stata Corp. College Station, Texas 77845 USA) where it was analyzed accordingly. The sero-prevalence of the disease for animal level was calculated on the basis of combined RBPT and CFT positivity, dividing the number of camels found to be seropositive for Brucella infection by the total number of tested camels. Herd level seroprevalence was calculated based on the number of herds positive by both RBT and CFT are combined divided by total number of herds. First univariable logistic regression analysis was employed to determine the associations of risk factors with sero-prevalance of camel brucellosis in the study areas. Those risk factors with $P<0.25$ in univariable logistic regression analysis were further analyzed using multi variable logistic regression analysis. Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals. Statistical significance is declared if the P value is $<0.05$.

# 4. RESULTS AND DISCUSSION

## 4.1. Seroprevalence of camel Brucellosis

According to the current study the overall sero-prevalence of camel brucellosis was 4.6% (18) (Table 4.1). Dividing the total number of positive herds by the total number of herds sampled, we found herd level seroprevalence of 6.7% (Table 4.1).

**Table 4.1.** Sero-prevalence of camel brucellosis using RBPT and CFT in Salahley region, Somaliland

| Location | N | RBPT No positive | *Sero-prevalence* % | CFT No positive | *Seroprevalence* % | *95%,CI* |
|---|---|---|---|---|---|---|
| Salahley | 140 | 15 | 10.7 | 4 | 2.8 | 0.007-.052 |
| Toon | 110 | 10 | 9 | 6 | 5.5 | 0.92- 10.57 |
| Kabada | 134 | 15 | 11.1 | 8 | 5.9 | 1.7-18.7 |
| Total | 384 | 40 | 10.4 | 18 | 4.6 | 0.02−0.068 |

N = number of camels examined; No. = number

The overall sero-prevalence of camel brucellosis was 4.6% This result is comparable to the previous reports of 4.1% (Hadush Angesom and Pal., 2013) in camels from Afar region of Ethiopia, 4.5% (Chauhan, *et al*., (2017) in camels from Gujarat, India and 4.4%, (Mohamed *et al.*, 2013) in camels of Abu Dhabi Emirate. Similarly it is in agreement with Mohamed *et al*., (2014) who reported 4.1% in Libya and Abbas and Agab (2002) who reported low seroprevalence (< 5%) in nomadic or extensively kept camels.

However, the current result was a litter higher than the observation of Habtamu Abafoge and Fisseha Moges (2014), who reported a seroprevalence of 3.67% in camel brucellosis from south Eastern of Tigray region, by Omer *et al.,* (2000) who reported, 3.1% in Eritrea, 1.5% by Robayo and Esubalew Chekol (2017) in Ethiopian Somali region, 0.9% in and around Dire Dawa by Gumi *et al*., (2013), 2.4% southeast Ethiopia by Berhanu Tilahun *et al.,* (2013) and 1.8% in Ethiopian Somali region and Borena by Bekele Megersa *et al.,* (2011).

Correspondingly, the result of the recent study was higher than that of Mohamed Abdurrahman *et al.,* (2011), who reported a lower seroprevalence of 1.6% in camels in and around Dire Dawa town. Furthermore the observed seroprevalence of this study was higher than that of Teshome Woldeab *et al.,* (2003) and Dominech, (1977), who reported a lower seroprevalence of 1.2%, 1.7%, and 1.7% from camels in Borana zone, Tigray region and Hararghe region, respectively.

On the other hand, the current study indicated a relatively lower seroprevalence of camel brucellosis that is 5.7% Teshome Woldeab *et al.,* (2003), 7.6%, Zewdu Mesfin and Hailleselassie Mekonnen (2012) in selected districts of Afar region and 5.4% Bekele Megersa *et al*., (2013) in a pastoral region. Moreover relatively, higher seroprevalence of camel brucellosis has been recorded 30.5% by Omer *et al*., (2007) in Sudan, 23.8% by Musa *et al.*, (2008) in Darfur, Western Sudan and 7.3% El-Boshy *et al.*, (2009) in Egypt.

The difference in seroprevalence among the current and previous studies might be due to the agro ecological differences of the study areas, sample size, animal management and production systems and the serological diagnostic tests used. Prevalence of brucellosis can vary according to climatic conditions, geography, species, sex, age and diagnostic tests applied (Gul and Khan, 2007). The movement of animals may worsen the epizootic situation of brucellosis in the study area as the disease spread from one herd to another due to the movement of an infected camel in to a susceptible camel herd (Radostitis *et al*., 2007).

In afar region mixing of the animals from various areas is common at communal grazing and watering areas (Teshale Sori *et al.,* 2006) while in Somaliland only animals belonging to a given clan are allowed to be mixed and there is a strong clan-based segregation of animals and use of rangeland. Additionally, the good practice of herders timely culling of aborted and non-conceiving females from the herds might have contributed to the situation.

This is true for Somaliland pastoral community in case of long dry seasons, because camel herders move from place to place for searching of feed and water to their animals. The difference in specificity and sensitivity of the serological testes applied may have also an effect on the higher seroprevalence result of this study. Higher seroprevalence of camel brucellosis might be recorded using tests that had poor specificity (Andreani *et al.,* 1982).

**Table 4.2** Herd level sero-prevalence of camel brucellosis using RBPT and CFT in Salahley region, Somaliland

| Location | N | RBPT No positive | *Sero-prevalence* % | CFT No positive | *Seroprevalence* % |
|---|---|---|---|---|---|
| Salahley | 112 | 13 | 11.6 | 9 | 8.03 |
| Toon | 123 | 17 | 13.8 | 10 | 8.1 |
| Kabada | 149 | 10 | 6.7 | 7 | 4.6 |
| Total | 384 | 40 | 32.1 | 26 | 6.7% |

N = number of herds examined; No. = number

## 4.2 Univariable and multivariable logistic regression analysis

### *4.2.1 Univariable and multivariable logistic regression analysis*

According to individual animal level univariable logistic regression analysis of potential risk factors such as sex, age, heard size, Interaction of camels with Other Ruminants(ICOR), Abortion in Female Adult Camels(AFAC) were found significant association with Brucella seropositivity (P<0.05). Risk factors such as district only Kabada is significantly different from Toon and Salahlay district (P<0.05) and single parity females were significantly different from multiparous adult female camels and females with no history of parturition (P< 0.05) (Table 4.3).

**Table 4**.3 Univariable logistic regression analysis of individual camel brucellosis in relation to Different risk factors in Salahley region, Somaliland

| Variables | Categories | No. sera Tested | No. sera Positive | Prevalence % | OR | P-value |
|---|---|---|---|---|---|---|
| **Sex** | Female | 234 | 16 | 6.8 | | |
| | Male | 150 | 2 | 1.3 | 0.23 | 0.05* |
| **Age** | ≤4 years | 134 | 2 | 1.4 | **Ref** | **Ref** |
| | 5-7years | 150 | 9 | 6 | 5.2 | 0.03* |
| | >7 years | 100 | 7 | 7.1 | 6.0 | 0.02* |
| **Districts** | Salahlay | 140 | 4 | 2.8 | **Ref** | **Ref** |
| | Toon | 110 | 6 | 5.5 | 3.1 | 0.06 |
| | Kabada | 134 | 8 | 5.9 | 5.7 | 0.000** |
| **Parity** | No parturition | 62 | 2 | 3.2 | Ref | Ref |
| | Single parity | 154 | 10 | 12.9 | 5.6 | 0.02* |
| | Multiple parity | 168 | 6 | 3.3 | 1 | 0.96 |
| | | 384 | | | | |
| **Herd size** | Small herd | 200 | 3 | 1.5 | Ref | Ref |
| | Medium herd | 84 | 5 | 5.9 | 10.9 | 0.000** |
| | Large herd | 100 | 10 | 10.5 | 12.3 | 0.000** |
| **ICWR** | Yes | 204 | 16 | 7.8 | 10.7 | 0.02* |
| | No | 180 | 2 | 1.1 | | |
| **AFAC** | Yes | 129 | 10 | 15.15 | 6.86 | 0.000** |
| | No | 255 | 8 | 3.13 | **Ref** | **Ref** |
| | | 384 | | | | |

*= Significance, **= strongly significance

No= Number; ICWR= Interaction of camels with Other Ruminants

AFAC= Abortion in Female Adult Camels; OR= Odds Ratio;

Multivariable logistic regression analysis of risk factors showed that age, herd size and keeping camels closely together with other ruminants as the major risk factors for of sero-positivity to Brucella infection in camels ($p < 0.05$) (Table 4.3 &4.4). Advance in age, herd sizes and keeping camels together with other ruminants were significantly associated with infection rate ($p < 0.05$)

when the putative effects of different factors subjected to step wise backward reduction method. Table.4.4, shows that increasing age and herd size together with keeping camels closely with other ruminants and abortion in female adult camels had significantly joint effect on sero-positivity in dromedaries when other factors removed ($p<0.05$) Thus, they were found to be the risk factors for the occurrence of camel brucellosis in the study area (Table 4.3 &4.4)..

**Age**

Infection may occur in animals of all age groups, but persists commonly in sexually mature animals of both sexes. The present study revealed the highest seroprevalence of camel brucellosis was in >7 year age groups with seroprevalence of 7.1% than 5-7 year with seroprevalence of 6.0% and < 4 year age groups with 1.4% seroprevalence. The difference in seroprevalence was also statistically significant in both univariable and multivariable logistic regression analysis where camels with >7 years old had 6 times and 5-7 years old had 5.2 times at higher risk of developing seroprevalence of brucellosis than animals with an age of < 4 years old (Table 4.3 &4.4).

This finding is in agreement with the observation of Habtamu Tasfya and Fisseha Girmatsion (2014), who reported a significantly higher occurrence of brucellosis(6.5%) in adult camels (>4 years) than (0%) young camels (6 month to 4 years) with a likelihood odds ratio (OR) of 9.6, from Mehoni district, south eastern of Tigray region, Ethiopia. the current study was also in agreement with Madu *et al*., (2016) who reported 16.7% in adult and 0.6% in young camels with $p< 0.05$, in three abattoirs from northern Nigeria.

This higher seroprevalence of brucellosis in older camels was in line with previous reports of Radostits *et al*., (2007) who indicated that infection may occur in animals of all age groups but persists commonly in sexually mature animals. On the other hand younger animals are more resistant to infection and frequently clear an established infection, although latent infection can occur (Walker, 1999). This may result from the fact that sex hormones and Erythritol which stimulate the growth and multiplication of Brucella organisms tend to increase in concentration with the age and sexual maturity (Radostitis *et al*., 2007).

**Herd size**

Brucellosis is considered as a disease of herd importance. The current study disclosed that large herd size had considerably high level seropositivity to brucellosis (10.5%) than medium (5.9%), and small, herd sizes (1.5%). There was also statistically highly significant difference in

seroprevalence among the three herd categories ($p<0.05$) in both univariable and multivariable logistic regression analysis. Large sized and medium sized herds had 12.4 and 10.9 times at higher risk of being seroreactors compared to small herd sized camels (Table 4.3 &4.4).

Different authors reported that herd size (small: 14 - 20, medium: 21- 40 and large: > 40 camels) showed statistically significant difference ($\chi 2 = 8.47$, $P = 0.004$) in the occurrence of the disease. Likewise Mohamed *et al.,* (2015) had observed the same effect of herd size for seroprevalence of camel brucellosis in Khartoum state Sudan and stated that multivariable analysis indicated that herd size comprising more than 20 camels was significantly associated with seroprevalence of camel brucellosis by logistic regression analysis (OR=5.7) with $P<0.05$. Similar association, was recorded by Bekele Megersa *et al*., (2013) OR= 3.05 with $p = 0.01$, in Afar region, North Eastern of Ethiopia and Adamu Mohamed r *et al*., (2014) OR= 7.8 with P=0.0003 in North Eastern Nigeria. Also Mohamed *et al*., (2013), who reported 4.4% ($p = 0.000$) seroprevalence of camel Brucellosis in Abu Dhabi. Similarly, Sisay Weldegebriel Zewold & Mekonnen Haileselassie (2012) shown that herd size was highly related to the brucella occurrence among camels in the area.

As herd size increases, the chance of contact between animals increase leading to more chances of infection which is particularly more important during calving or abortion when most of brucellosis contamination occur (Mohamed *et al.,* 2013).Therefore, herd size and density of animal population together with poor management are directly related to infection rate (Abbas and Agab, 2002). Herd size is documented by Radostits *et al*., (2000), as a main factor for transmission of Brucella infection.

**Interaction of camel with other ruminants (**ICOR)

The present study revealed that camels interacting with other ruminants had higher seroprevalence of brucellosis (7.8%) than those camels not interacting (1.1%) with other ruminants. In both univariable and multivariable logistic regression analysis there was significant difference in seroprevalence of brucellosis ($P<0.05$) (Table 4.3 &4.4).

Also Chauhan, *et al*., (2017) observed that rearing of multispecies in same herd may lead to close contact of animals, which may facilitate the exchange of various pathogenic microorganisms. In the present study area, higher seroprevalence was observed in camels reared with small ruminants which might be the possible source of infection for camel. Similarly, Abou-Eisha (2000), observed high seroprevalence in camels with a history of sheep and goats

being kept together (with the camels). This may have shown comparable results had it been included in this study about the comparison of the species seroprevalence.

Most species of Brucella are primarily associated with certain hosts; however, infections can also occur in other species, particularly when they are kept in close contact. Most of the pastoral community in the study area, keep camels together with other ruminants while browsing, watering, in night enclosures and during migration, which might create an opportunity for the inter species transmission of the disease. This reflect the real situation of brucellosis among the two groups which pay the attention to study the role of ruminants ( sheep, goats and cattle ) in transmitting brucellosis to camels and vice versa. Factors that contribute to this high prevalence in camels may be related to the extensive management system livestock prevailing in the study area. According to the current result of the questionnaire survey 82% (37/45), of the respondents keep camels closely together with other ruminants.

**Abortion in Female Adult Camels**

According to univariable logistic regression analysis female camels with history of abortion (15.15%) were found to be significant seroreactors than female camels with no history of abortion (3.13%) with (p=0.000). It was also evident that female camels with history of abortion were 6.86 times more likely at risk of being seroreactors to brucellosis than those female camels with no history of abortion (Table 4.2 &4.3).

Past study indicate that dromedaries camels that live in flocks with the history of abortion were 2.7% times more likely to be infected than animals in flocks without the history of abortion, which is in agreement with previous reports (Fatima et al., 2016; Ismail et al., 2012; Musa and Shigidi, 2001). Abortion has been reported in pregnant camels infected with brucellosis in a study done at south Algeria in 2021.

**Table 4.4** Multivariable stepwise logistic regression analysis of risk factors (sex, age, districts, Herd size, ICOR) for sero-prevalence of camel brucellosis in Salahley region

| Variables | | No. sera Tested | No. sera Positive | Crude OR | Adjusted OR | P-value |
|---|---|---|---|---|---|---|
| **Sex** | Female | 234 | 16 | | | |
| | Male | 150 | 2 | 0.23 | 0.30 | 0.138 |
| **Age** | ≤4 years | 134 | 2 | - | - | - |
| | 5-7years | 150 | 9 | 5.3 | 3.14 | 0.003* |
| | >7 years | 100 | 7 | 6.0 | - | - |
| **Districts** | Salahlay | 140 | 4 | - | - | - |
| | Toon | 110 | 6 | 3.1 | 1.02 | 0.952 |
| | Kabada | 134 | 8 | 5.9 | - | - |
| **Herd size** | Small herd | 200 | 3 | - | - | - |
| | Medium | 84 | 5 | 10.7 | 4.37 | 0.000** |
| | Large herd | 100 | 10 | 15 | - | - |
| **ICOR** | Yes | 234 | 24 | 10.2 | 11.8 | 0.020* |
| | No | 150 | 1 | - | | |
| **AFAC** | Yes | 66 | i10 | 15.15 | 6.86 | 0.000** |
| | No | 255 | 8 | 3.13 | **Ref** | **Ref** |

*= significant, **= strongly significant ***= very strongly significant No= Number; ICOR= Interaction of camels with Other Ruminants, OR= Odds ratio

## 4.3 Questionnaire

### *4.3.1 Sociodemography & educational status*

**Gender**

The current questionnaire survey revealed that the majority of camel owners were male (92%). (Table 4.5).In agreement with the current study Fekadu Gutema *et al.* (2021) reported that the majority of (79.2%) of respondents were male headed house hold.

Similarly in agreement with a research work conducted in Malaysia showed that males are at greater risk of contracting brucellosis since they are commonly involved in the handling of livestock and consume uncooked animal product, especially in the pastoral area (Ahmad, and Hashim, 2015).

**Age of respondents**

In this study it was apparent that most respondents (51.5%) were under the age group 26-45year (Table 4.5). Similarly, Fekadu Gutema *et al.* (2021) reported that the majority of (65.8%) of respondents were in the age range of 25-59 year. Our results are in agreement with a study in Bangladesh where individuals of age group 40–80 years were more likely to be infected with brucellosis (Rahman AK *et al* 2012). Another study in Lebanon reported that brucellosis cases increased with age group (Kalaajieh WK *et al* 2015).

**Marital status**

According to this questionnaire survey the majority of camel owners **(63.5%)** involved in the questionnaire survey were married (Table 4.4).

In agreement with the current study Fekadu Gutema *et al.* (2021) reported that the majority of (85 %) of respondents were married. Similarly Philip Bobu igawe *et al.* (2020) in Abuja, Nigeria showed the Most of the respondents were married (73.2%).

Contrary to this Charity Ashe'osla Agada *et al.* (2018) around Nigeria found Married (8.94%) and single (13.51%), the difference may be due to the age of the target of respondents, methodological uses of interview and cultural norms of society.

**Education Level**

The current questionnaire survey revealed that more than half of the respondents (56.5%) were illiterate (Table 4.5). In agreement with the current study Fekadu Gutema *et al.* (2021) reported that the majority of (67.5 %) of respondents were illiterate. However, the study conducted in Kenya showed a high level of knowledge of brucellosis in pastoral communities where respondents testified brucellosis to be a zoonotic disease and abortion as its common indicator (Obonyo and Gufu, 2013). This might be due to the difference in the educational access and coverage in the pastoral area of the two countries.

**Table 4.5** Sociodemographic & educational status of camel owners in Sahaley region, Somaliland

| Variables | Frequency(60) | Percentage (%) |
|---|---|---|
| **Gender** | | |
| Male | 55 | 92% |
| Female | 5 | 8% |
| **Age of respondents** | | |
| 10-25 | 10 | 16.5% |
| 26-45 | 31 | 51.5% |
| 46 and above | 19 | 32% |
| **Marital status** | | |
| Single | 22 | 36.5% |
| Married | 38 | 63.5% |
| **Education Level** | | |
| Illiterate | 34 | 56.5% |
| Primary school | 10 | 16.5% |
| Secondary and above | 16 | 27% |

### 4.3.2 Production system & economic importance

**Production system & grazing system**

In the current study questionnaire survey analysis showed that 80% of the management system is extensive management system; whereas the rest 20% camels were kept intensive management system (Table 4.6). In this study 45% and 55% of camel owners were grazing their camels separately and together with other ruminants, respectively. On the other hand, majority of camel herds (78%) had separate night resting area for their camel (Table 4.6).

Comparable result was reported by Fekadu Gutema *et al.* (2021) where 78% of the respondents mix their camel at gazing point. It is known that the risk of the disease is higher in intensively reared animals due to increased exposure (Gwida et al 2012), and in this study, all sampled camels were from extensive kept dairy camels, to which this relatively higher prevalence might be attributed.

**Source of water**

As per the respondents, during dry season, Camel owners in the study area were using traditional wells (71%) and ponds (29%) made by the community, Somaliland government, traditional wells is the main water sources for their camels.

Similarly (Wolde 1991; Coppock 1994) reported that the Wells, ponds and rivers are the main sources of water for camels in Ethiopia. Watering animals from the deep wells is an arduous dry-season activity which is the responsibility of mainly young men, but it is also common to see older youths of both sexes involved (Coppock 1994). The watering sites are usually visited by large numbers of camels and other animals at a time from the surrounding as well as from distant areas. Mostly the pond and river water sources are shared by wild animals.

**Use of camel**

According to the respondents camels are mainly reared for either milk or meat or both production (98%) (Table 4.6). In the study area, 75% of the total milk production from Salahlay and Toon was sold to the private milk collecting centers, which were established in Hawd Centre, 35kms to the west of Hargiesa city, where later taken to Hargeisa town, capital city of Somaliland to generate income. The remaining 25% milk was used for home consumption.

In agreement with Bornstein (2010) where camels are used for food (milk and meat) and textiles (fiber and felt from hair). Camels are working animals especially suited to their desert habitat and are a vital means of transport for passengers and cargo.

**Source of bull**

According to the respondent's majority (55%) of camel herders were using breeding bull from their own herd. Only 45% of the herders were using communal village bull (Table 4. 6).

In agreement with the current study indicates that pastoralists highly value and consider milk production potential of camels as evidenced by their trait preferences (Tadesse et al. 2014b), proportion of female camels they keep in the herd (Megersa et al. 2008; Ahmed Shek et al. 2005 a, b) and their bull selection practices (Wolde 1991; Tezera and Belay 2002).

It is a common practice for pastoralists to keep higher number of female camels than males at all age categories, i.e. calves, growing young ones and adults (Tezera and Belay 2002; Bekele & Kibebew 2002), indicating the importance of reproduction & milk production in arid areas.

**Method of disposal of aborted fetus & placenta**

Sixty five percent of the respondents mentioned that aborted fetus, placenta and discharges were either left on the ground and 35% threw to the dogs of the herders (Table 4.6). Comparable result was reported by Fekadu Gutema *et al.* (2021) where 81.37% of the respondent's disposal of the placental and aborted fetus in the open field.

Similarly, Study conduct by (Eshetu et al., 2018) Regarding the management of aborted fetal membrane/aborted fetus and discharge, 83(81.37%) of the respondent told that, they throw it on the field and some of0 the respondents (4.9%) practiced proper disposal of the aborted fetal materials.

**Table 4.6.** Production system and economic importance of camel in Sahaley region, Somaliland

| Variable | frequency | Percentage |
|---|---|---|
| **Production system** | | |
| Extensive | 48 | 80% |
| Intensive | 12 | 20% |
| **Keeping Camel with other animals** | | |
| Kept with other animals | 38 | 63% |
| Kept alone | 22 | 37% |
| **source of Water** | | |
| Well | 43 | 71% |
| Pond | 17 | 29% |
| **Camel uses** | | |
| All productions | 59 | 98% |
| Milk production/meat | 35 | 58% |
| Transport | 1 | 2% |
| Both | 24 | 40% |
| **Source of bull** | | |
| Uncommon | 27 | 45% |
| Own bull | 33 | 55% |
| **Method of disposal of placenta & aborted fetus** | | |
| Left on the ground | 39 | 65% |
| Given to dogs | 21 | 35% |

## 4.4 public health importance

Brucellosis is a public health hazard, and there are high risk humans other than occupational contactors particularly to pastoral households who in many ways are exposed to the disease either through consumption of raw milk or milk products of seropositive animals and deliberately handling of potentially infected animals particularly in cases of grooming animals an assisting she camels for parturition process.

Based on the questionnaire results of this study almost all (97%) camel owners of the study area consume raw milk, and do delivery assistance, grooming livestock, clean newborns, assist suckling and carry the young from field to home without wearing any protection equipment and or materials (Table 4.7). Also people in the study area consume milk after mixing with boiled tea so called “Caddeys” in Somali language.

Comparable result was reported by Fekadu Gutema *et al.* (2021) where 91.7% of the respondents drink raw milk. Similarly, Mohamed *et al.*, (2013) reported that all the herders (100%) from Buroa districts in Somaliland consumed fresh raw milk without any heat treatment.

According to the respondents view Camel meat is consumed in cooked form. However, some of the respondents said that they consume the hump of camel in raw. Comparable to this study Fekadu Gutema *et al.* (2021) report 78.33% of the respondents consume camel meat after heating

The majority of camel owners (63%) touch aborted fetus and placenta without wearing any protective hand gloves. In agreement to the present study Fekadu Gutema *et al.* (2021) reported that 90% of the respondents remove aborted fetus and placenta without protective gloves.

According to the current study 97% of the respondents did not have knowledge about brucellosis (Table 4.7). In agreement with our study Fekadu Gutema *et al.* (2021) reported that 90% of respondents don’t know about brucellosis. Similarly, Habtamu and Fisseha (2014), reported from Mehoni district in south eastern part of Tigray region, that most animal owners were not aware of the zoonotic nature of brucellosis, as they drank raw milk and did not take precautions in handling aborted fetuses.

**Table 4.7.** Public health importance of camel brucellosis in Sahaley region, Somaliland

| Milk used to drink | Number | Percent |
|---|---|---|
| Raw milk | 58 | 97% |
| Boiled milk | 2 | 3% |
| **Touching of aborted Materials/fetus** | | |
| Yes | 38 | 63% |
| No | 22 | 37% |
| **Knowledge of brucellosis** | | |
| Yes | 2 | 3% |
| No | 58 | 97% |

## 4.5 Seroprevalence of human brucellosis in study area

Out of the 60 serum samples taken from camel owners the overall prevalence of human brucellosis was 2(3.3%) (Table 4.8). The CFT test revealed that Toon and Salahley were the districts with relatively higher prevalence of human brucellosis with a proportion of 5%.. The difference in seroprevalence between the current and previous studies might be due to the agro ecological differences of the study areas, sample size, animal management and production systems. Prevalence of brucellosis can vary according to climatic conditions, geography, sex, age and diagnostic tests applied (Gul and Khan, 2007). The movement of animals may worsen the epizootic situation of brucellosis in the study area as the disease spread from one herd to another due to the movement of an infected camel in to a susceptible camel herd (Radostitis *et al*., 2007).

Meanwhile, the seroprevalence was higher in female (37.6%) and in adult 8(22.8%). Higher seroprevalence of human brucellosis were reported in some districts of Ethiopian-Somali with seroprevalence of 2.4% using RBPT but 0.4% using CFT for Brucella antibody indicating public health importance the disease among pastoral communities in the area.

The sero-prevalence obtained is higher than the 2.2% by Mekonnen, Shewit, Moses, Mekonnen, and Kelay Belihu (2011), 1.2% by Tibesso Ibrahim and Tadele Tolosa (2014), but slightly lower than 3.4% by Tadele Tolosa (2004) around Jima, 5.8% by Kassahun Asmare (2004) Sidama area and 3.8% by Mussie Hailemelekot *et al*. (2007) around Bahir dar, 5.5% & 6.4% Chuku

1985 in Nigeria & Uganda, respectively and 4.6% Omer et al 2002 in Eritrea.

Our study on seroprevalence of human brucellosis showed more femalesmales (4.7%) seroreactors compared to fmales (2.6%) (Table 4.8). in agreemnet to this Agasthya *et at.* (2007) around Jammu, India, reported 98.6% prevalence in males as compared to 1.03% in females by using I-ELISA. This may associated with the fact that males are involved in assisting delivery and removal of placenta. Contrary to this Mussie Hailemelekot *et al.* (2007) around Bahir dar found females and males were equally susceptible.

In this study it was apparent that the seroprevalence of brucellosis was higher in young (4%) than young than adults (2.*%) (Table 4.8). contrary to this study Mussie Hailemelekot *et al*. (2007) around Bahir dar reported that old age & middle age groups are more affected than their young counter parts frequent and long period of contact might contrinute the effect.

**Table 4.8** Sero prevalence of human brucellosis in Sahaley region, Somaliland

| Variables | Categories | No. sera Tested | No. RBPT Positive% | No. CFT Positive% |
|---|---|---|---|---|
| **Sex** | Female | 39 | 4(10.2) | 1(2.6) |
| | Male | 21 | 1(4.7) | 1(4.7) |
| | Total | 60 | 5 | 2(3.33%) |
| **Age** | Young | 25 | 2(8) | 1(4) |
| | Adult | 35 | 3(8.6) | 1(2.8) |
| | Total | 60 | 5 | 2(3.3%) |
| **Districts** | Salahlay | 20 | 2(10) | 1(5) |
| | Toon | 20 | 2(10) | 1(5) |
| | Kabada | 20 | 1(5) | 0(0.0) |
| | Total | 60 | 5(8.3) | 2(3.3%) |

# 6. CONCLUSION AND RECOMMENDATIONS

According to the current study the overall individual level sero-prevalence of camel brucellosis was 4.6% and herd level sero prevalence of camel brucellosis was 6.7%. Multivariable logistic regression analysis of presumed risk factors indicated that age, herd size, previous history of abortion, and camels closely kept with other ruminants were the major associated risk factors for the occurrence and transmission of camel brucellosis in the study areas. The existence of the disease together with the extensive production systems and practices including livestock movements, sharing of grazing grounds and watering points, mixing and trading of animals that were prevailing in the study area intensify the condition and increase the prevalence of brucellosis. The seroprevalence of human brucellosis in the study areas was 3.3%. the livelihood of pastoral community is mainly dependent on camel, providing milk and meat. Furthermore lack of awareness about zoonotic diseases, habit of raw milk consumption and close contact with animals was highly common in the study area. Therefore the results of the current study provide the importance of camel brucellosis in selected districts.

Based on the above conclusion the following recommendations are forwarded:-

- Further epidemiological studies involving the role of other ruminants for the occurrence of camel brucellosis and transmission of the disease in pastoral areas leading to improvement of health and management of camels is greatly essential.
    - Awareness creation through Public health educational programmes on recent animal husbandry and management systems of animal diseases and risk of zoonotic diseases including brucella is highly recommended.
    - In order to design and implement control measures through test & slaughter & vaccination programmes aiming at preventing further spread of camel brucellosis in study area, advanced research work with more emphasis on the isolation and identification of the Brucella biotypes circulating in camels in the study area is crucial.

## REFERENCE


Abbas B. and Agab H. 2002. A review of camel Brucellosis. Preventive Veterinary Medicine, 55: 47-56.

Abou-Eisha A.M., 2000. Brucellosis in camels and its relation to public health. Assiut Veterinary Medical Journal, 44(87): 54-64.

Agada, C. A. O., Mohammed, J., Okoh, A. E. J., & Ogugua, J. A. (2018). Prevalence and risk factors associated with brucellosis among high-risk individuals in Lafia, Nasarawa state, Nigeria. International Journal of One Health, 4(8), 45-51.

Agasthya A.S, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. Indian J. Med. Microbiol. 2007;25:28–3.

Ahmad, S., Yaqoob, M., Hashmi, N., Ahmad, S., Zaman, M. A., & Tariq, M. (2010). Economic importance of camel: Unique alternative under crisis. Pakistan Veterinary Journal, 30(4), 191-197.

Ahmadi N.A., 2005. Hydatidosis in camels (Camelus dromedarius) and their potential role in the epidemiology of Echinococcus granulosus in Iran. Journal of helminthology, (2): 119.

Alamian S., Dadar M., 2019. Brucella abortus contamination of camel milk in two Iranian regions. Prev. Vet. Med., 169:104708

Alegbeleye, Oluwadara Oluwaseun, Ian Singleton, and Anderson S. Sant'Ana. "Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review." Food microbiology 73 (2018): 177-208.

Al-Majali A.M., Talafha A.Q., Ababneh M.M. and Ababneh M.M., 2009. Seroprevalence and risk factors for bovine brucellosis in Jordan. Journal of Veterinary Science, 10(1): 61-65.

Alshwany E.A.A., 2019. The epidemiology of Brucellosis in sheep, goats and humans in the Iraqi Kurdistan Region (Doctoral dissertation, Murdoch University).

Alton G.G., Jones M.J., Lois M. and Peitz D.E. 2010. Serological methods .In: Laboratory Techniques in brucellosis.2nd ed. WHO, Geneva.pp.64-124.

Balako Gumi , Rebuma Firdessa , Lawrence Yamuah , Teshale Sori , Tadele Tolosa , Abraham Aseffa , Jakob Zinsstag , Esther Schelling, 2013. Seroprevalence of brucellosis and Q-fever in southeast Ethiopian pastoral livestock. Journal of veterinary science & medical

diagnosis, 2(1):10.

Bamaiyi P.H., Hassan L., Khairani-Bejo S., ZainalAbidin M., Ramlan M., Krishnan N., Adzhar A., Abdullah N., Hamidah N.H.M., Norsuhanna M.M. and Hashim S.N., 2014. Case–control study on risk factors associated with Brucella Melitensis in goat farms in Peninsular Malaysia. Tropical animal health and production, 46(5): 739-745.

Banai M. and Corbel M. 2010. Taxonomy of Brucella. Open Veterinary Science Journal. 4:

Bekele Megersa, Demelash Biffa, Fufa Abunna, Alemayehu Regassa, Godfroid J. and Skjerve E., 2012. sero epidemiological study of livestock brucellosis in a pastoral region. Epidemiology & Infection, 140(5): 887-896.

Berhanu Tilahun, Merga Bekana, Kalay Belihu, and Endrias Zewdu. 2013. Camel brucellosis and management practices in Jijiga and Babile districts, eastern Ethiopia. Journal of Veterinary Medicine and Animal Health, 5(3): 81-86.

Berhanu Tilahun,, Merga Bekana, Kalay Belihu and Endrias Zewdu (2013): Camel Brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia. Journal of Veterinary Medicine and Animal Health., 5(3):81-86.

Blasco J.M. and Molina-Flores B., 2011. Control and eradication of Brucella melitensis infection in sheep and goats. Veterinary Clinics: Food Animal Practice, 27(1): 95-104.

Bornstein, Set (2010). "Important ectoparasites of Alpaca (Vicugna pacos)". Acta Veterinaria Scandinavica. 52 (Suppl 1): S17. doi:10.1186/1751-0147-52-S1-S17. ISSN 1751-0147. PMC 2994293.

Cook D.R. and Kingston G.C., 1988. Isolation of Brucella suis biotype 1 from a horse. Australian veterinary journal, 65(5): 162-163.

Coppock, D.L. 1994. The Borana plateau of southern Ethiopia: Synthesis of pastoral research, development and change, 1980–91, 374. Addis Ababa: ILCA systems study no. 5, International Livestock Centre for Africa.

Corbel M.J., 2006. Brucellosis in humans and animals. World Health Organization. pp 89.

Damir H.A., Tageldin M.H., Kenyon S.J. and Idris O.F., 2021. Isolation of Brucella abortus from experimentally infected dromedary camels in Sudan: a preliminary report. Veterinary Research Communications, 13(6): 403-406.

Daneshvar N., Salari D. and Khataee A.R., 2003. Photocatalytic degradation of azo dye acid red 14 in water: investigation of the effect of operational parameters. Journal of Photochemistry and Photobiology A: Chemistry, 157(1): 111-116.

Danner G.R. and Merrill P., 2006. Disinfectants, disinfection and biosecurity in aquaculture. Aquaculture biosecurity: Prevention, control, and eradication of aquatic animal disease, 91-128.

El-Amier Y.A., Elnaggar A.A. and El-Alfy M.A., 2017. Evaluation and mapping spatial distribution of bottom sediment heavy metal contamination in Burullus Lake, Egypt. Egyptian Journal of Basic and Applied Sciences, 4(1): 55-66.

El-Radhi A.S., 2018. Fever in Common Infectious Diseases. In: Clinical Manual of Fever in Children (pp. 85-140). Springer, Cham.

Emmerzaal A., De Wit J.J., Dijkstra T., Bakker D. and Van Zijderveld F.G., 2002. The Dutch Brucella abortus monitoring programme for cattle: The impact of false-positive serological reactions and comparison of serological tests. Veterinary quarterly, 24(1): 40-46.

Eshetu, A., Belina, D., Jafer, M., Mengistu, S., 2018. Sero-Prevalence Of Brucellosis In Camels And Febrile Human Patients Attending Health Facilities In Selected Districts Of Eastern Ethiopia.

Esubalew Solomon. 2017. Review of ethnobotanical and ethnopharmacological evidences of some Ethiopian medicinal plants traditionally used for the treatment of cancer." Ethiopian Journal of Health Development 31(3): 161-187

Fekadu Gutema, Kebede Amenu, Adugna Chalchisa and Gezahegne Mamo Brucellosis in 2021. Camels and Humans: Seroprevalence and Associated Risk Factors in Amibara District of Afar Region, Ethiopia, Veterinary Medicine International, 5482725: 10

Fowler M.E. 2010. Medicine and surgery of camelids, 3rd Ed. Wiley-Blackwell, 207–208.

Garin-Bastuji B., Blasco J.M., Marin C. and Albert D., 2006. The diagnosis of brucellosis in sheep and goats, old and new tools. Small Ruminant Research, 62(1-2): 63-70.

Gessese, A. T., et al. "Seroprevalence of brucellosis in camels (Camelus dromedaries) in South East Ethiopia." Journal of Veterinary Scientific Medical Diagnosis 3.1 (2014): 2.

Głowacka, Patrycja, et al. "–Virulence Factors, Pathogenesis and Treatment." Polish journal of microbiology 67.2 (2018): 151-161.

Godfroid J., Nielsen K. and Saegerman C., 2010. Diagnosis of brucellosis in livestock and wildlife. Croatian medical journal, 51(4):296-305.

Godfroid, Jacques, et al. "Brucellosis in terrestrial wildlife." Revue Scientifique et Technique. Office International des Epizooties (2013).

Greenfield M.D., 2002. Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford University Press. PP

Gul, S. T., & Khan, A. (2007). Epidemiology and epizootology of brucellosis: A review. Pakistan veterinary journal, 27(3), 145.

Gwida M., El-Gohary A., Melzer F., Khan I., Rösler U. and Neubauer H., 2012. Brucellosis in camels. Research in veterinary science, 92(3): 351-355.

Gwida M.M., El-Gohary A.H., Melzer F., Tomaso H., Rösler U., Wernery U., Wernery R., Elschner M.C., Khan I., Eickhoff M. and Schöner D., 2011. Comparison of diagnostic tests for the detection of Brucella spp. in camel sera. BMC research notes, 4: 525.

Habtamu Tassew, Richard B., Dana H., Kassaw A. T., 2015. Camel Brucellosis: Its Public Health and Economic Impact in Pastoralists, Mehoni District, Southeastern Tigray, Ethiopia. Journal of Microbiology Research, 5(5): 149-156.

Hadush Angesom and Mahendra Pal1, 2013. Seroepidemiology of camel brucellosis in the Afar region of Northeast Ethiopia. Journal of Veterinary Medicine and Animal Health 5(9): 269-275.

Higgins AT, Allen WR, Mayhew IG, Snow DH, Wode J. 2002. An Introduction to the camel in health and disease in proceeding of the first International camel conference. In: R, and W Publications UD, London, new market. ARC Onderstepoort, Berg En-Dal, South Africa: OIE Internationa Congress. 2002; 49.

Hotam Singh Chaudhary and Akriti Srivastava, 2011.Brucellosis: Its Diagnosis, Prevention and Treatment. J. Chem. Pharm. Res., 3(6): 912-917.

Isam M., 2016. Prevention of Camel Brucellosis Spreading to Humans: A Real Challenge. EC Bacteriology and Virology Research 2.1: 36.

Jay, Maryne, et al. "Serological, cultural and molecular evidence of Brucella melitensis infection in goats in Al Jabal Al Akhdar, Sultanate of Oman." (2018).

Kalimuddin S, Seow CJ, Barkham T, Deepak RN, Li L, Tan TT. 2010. Hidden health risks of the Hajja report of two cases of brucellosis contracted by pilgrims during the Hajj. Scand J Infect Dis. 42: 228–230.

Kebede T, Getahun Ejeta and Gobena Ameni, 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Médecine Vétérinaire, 159(1): 101.

Khan Gul Majid and Victor M. Meidan. 2007. Drug release kinetics from tablet matrices based upon ethylcellulose ether-derivatives: A comparison between different formulations." Drug Development and industrial pharmacy, 33 (6): 627-639.

Köhler-Rollefson I, Mundy P, Mathias E. A. 2001. Field Manual of Camel Diseases. Traditional and Modern Health Cares for Dromedaries. London: ITDG publ.; 253.

Larsson P.G., Bergström M., Forsum U., Jacobsson B., Strand A. and Wölner-Hanssen P., 2005. Bacterial vaginosis Transmission, role in genital tract infection and pregnancy outcome: an enigma: Review article III. Apmis, 113(4): 233-245.

Liu M., 2015. Brucella. In: Molecular Medical Microbiology. Academic Press. pp. 1781-1788.

López-Goñi I., García-Yoldi, D., Marín C.M., de Miguel M.J., Barquero-Calvo E., Guzmán-Verri C., Albert D. and Garin-Bastuji B., 2011. New Bruce-ladder multiplex PCR assay for the biovar typing of Brucella suis and the discrimination of Brucella suis and Brucella canis. Veterinary microbiology, 154(1-2): 152-155.

Madu G. A., Adama O. R., James B. W., Hassan M., Lubabatu I. Esther M., 2016. Sero-prevalence of camel brucellosis in three abattoirs of Northern Nigeria. Journal of Veterinary Medicine and Animal Healt., 8(3):15-20.

Mammeri A., Boukerche A. and Lu G., 2014, Lane detection and tracking system based on the MSER algorithm, hough transform and kalman filter. In: Proceedings of the 17th ACM international conference on Modeling, analysis and simulation of wireless and mobile systems (pp. 259-266).

Mariana (2009) the genus Brucella and clinical manifestations of brucellosis. The Open Veterinary Science Journal, 4: 71.

Marquez, Aurélie, et al. "Overview of laboratory methods to diagnose Leptospirosis and to identify and to type leptospires." International Microbiology 20.4 (2017): 184-193.

Megid J., Mathias L.A. and Robles C., 2010. Clinical manifestations of brucellosis in domestic animals and humans. The Open Veterinary Science Journal,(4): 119-126.

Mohammud Aden, Waktole, Hika, , and Hagos Ashenafi. "Seroepidemiology of camel brucellosis in and around Dire Dawa, Eastern Ethiopia." Veterinary Medicine International 2022 (2022).

Moraveji M., Hosseini A., Moghaddar N., Namavari M.M. and Eskandari M.H., 2012. Development of latex agglutination test with recombinant NcSAG1 for the rapid detection of antibodies to Neospora caninum in cattle. Veterinary parasitology, 189(2-4): 211-217.

Moreno E., 2014. Retrospective and prospective perspectives on zoonotic brucellosis. Frontiers in microbiology, 5: 213.

Mori Y., Tomita N., Kanda H. & Notomi T., 2012. Novel Molecular Diagnostic Platform for Tropical Infectious Diseases. Current Topics in Tropical Medicine, 445

Musa M.T. & Shigidi M.T.A. 2008. Brucellosis in camels in intensive animal breeding areas of Sudan. Implications in abortion and early-life infections. Rev. Elev. Méd. vét. Pays trop., 54 (1): 11–15.

OIE, 2000. Bovine brucellosis: Diagnostic Technique. Manual of Standard for Diagnostic Tests and Vaccines. 4th Ed. Paris: Office International des Epizootics 1-37.

OIE, 2008. Manual of Standard for Diagnostic Tests and Vaccines: Bovine Brucellosis. OIE, Paris, pp: 624-659.

Omer MM, Musa MT, Bakhiet MR, Perrett L. 2010. Brucellosis in camels, cattle, and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev Sci Tech., 29: 663–9.

PAHO/ WHO. 2001. Zoonoses and Communicable Diseases Common to Man and Animals. 3rd edition. V I. Bacteriosis and Mycosis. Scientific and Technical Publications. No 580. Pan American Health Organization Pan American Sanitary Bureau, Regional Office of the World Health Organization. Washington D.C. USA.

Parvizi J., Azzam K., Ghanem E., Austin M.S. and Rothman R.H., 2009. Periprosthetic infection due to resistant staphylococci: serious problems on the horizon. Clinical Orthopaedics and Related Research®, 467(7): 1732-1739.

Percin D., 2013. Microbiology of brucella. Recent patents on anti-infective drug discovery, 8(1): 13-17.

Plummer, Paul J., et al. "Management of C oxiella burnetii infection in livestock populations and the associated zoonotic risk: A consensus statement." Journal of veterinary internal medicine 32.5 (2018): 1481-1494.

Poester F., Nielsen K., Ernesto Samartino L. and Ling Yu W., 2010. Diagnosis of brucellosis. The Open Veterinary Science Journal, 4: 71.

Potter M.E., 2013. Brucellosis, Foodborne Infections and Intoxications. Elsevier Inc. 4th Edition.Pp:586.

Pulendran B., Li S. and Nakaya H.I., 2010. Systems vaccinology. Immunity, 33(4): 516-529.

Racloz V., Esther S., Nakul C., Roth F., & Jakob Z. 2013. Persistence of brucellosis in pastoral systems. OIE Revue Scientifique et Technique 32 (1): 61-70.

Radostits O.M., Gay C.C., Hinchcliff K.W. & Constable P.C. 2007. Veterinary medicine. A textbook of the diseases of cattle, horses, pigs and goats, 10th Ed. Saunders Elsevier, 963–994.

Radwan S.S., Al-Awadhi H. and El-Nemr I.M., 2020. Cropping as a phytoremediation practice for oily desert soil with reference to crop safety as food. International journal of phytoremediation, 2(4): 383-396.

Ramet, J-P. The technology of making cheese from camel milk (Camelus dromedarius). No. 113. Food & Agriculture Org., 2001.).

Roberts J.S. 1971. Veterinary Obstetrics and Genital Diseases. 2nd ed. India: CBS Publisher and Distributors, 108-112.

Robinson R. 2003. Guidelines for coordinated human and animal brucellosis surveillance.Food and Agriculture Organization of the United Nations. Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases. Emergency Prevention System, Food and Agriculture Organization of the United Nations.

Sadiq M. A., Ajogi I., Bale J. O. O., Mosimabale F. B., Tijjani A. N .and kaikabo A. A. 2011. Serological Survey of Antibodies against Brucella Organisms in One Humped Camel (Camelus dromedarius) Herds in the Lake Chad Area of Borno State, North Eastern Nigeria. Nigerian Veterinary Journal, 32 (1): 45 - 48.

Saegerman J., Nielsen K. and Godfroid C., 2010. Diagnosis of brucellosis in livestock and wildlife. Croatian medical journal, 51(4): 296-305.

Schmidt G.G., 2003. The Art and Artists of the Fifth Zionist Congress, 1901: Heralds of a New Age. Syracuse University Press.

Scholz, Godfroid, J.,, H. C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., ... & Letesson, J. J. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive veterinary medicine, 102(2), 118-131.

Schwarz, Norbert Georg, et al. "Microbiological laboratory diagnostics of neglected zoonotic diseases (NZDs)." Acta tropica 165 (2017): 40-65.

Sewell M.M.H. and Brocklesby D.W., 1990. Handbook on animal diseases in the tropics. Bailliere Tindall.

Shoukat, Shabu, et al. "Brucellosis: a current review update on zoonosis." J Immunol Immunopathol 19.2 (2017): 61-69.

Simister N.E., 2008. Placental transport of immunoglobulin G. Vaccine, 21(24): 3365-3369.

Sisay Weldegebriel Zewold, Mekonnen Haileselassie, 2012. Seroprevalence of brucella infection in camel and its public health significance in selected districts of Afar region, Ethiopia. Journal of Environmental and Occupational Science. 1(2): 91-98.

Smit S., 2013. Bovine brucellosis in Bangladesh: Estimation of true prevalence and diagnostic test-characteristics (Doctoral dissertation). Faculty of Bioscience Engineering, Ghent University, Belgium).

Sprague L.D., Al-Dahouk S. and Neubauer H., 2012. A review on camel brucellosis: a zoonosis sustained by ignorance and indifference. Pathogens and global health, 106(3): 144-149.

STATA version 13.0 for windows (Stata Corp. College Station, Texas 77845 USA

Tadele Tolosa, 2004. Sero-prevalences study of bovine brucellosis and its public health significance in selected sites of Jimma Zone, Western Ethiopia. Ethiopia: Msc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.

Tanko M. and Dabo Z., 2013. Improving the content of auditors report as a means of fathom to audit expectation gap in Nigeria. International Review of Management and Business Research, 2(2): 580-598.

Teshale Sori, Muhie yimer, Abayneh Dagne and Awoke Kidanemarium, 2006. Seroprevalence of Small Ruminant Brucellosis in Selected Districts of Afar and Somali Pastoral Areas of eastern Ethiopia: the impact of Husbandry Practice. Revue Med. Vet. 157(11): 557-563.

Thakur, S. D., et al. "Marine mammal brucellosis: a new dimension to an old zoonosis." Current Science (2012): 902-910.

Than N. 2007. Prevalence Survey of Bovine Brucellosis (Brucella abortus) in Dairy Cattle in Yangon,Myanmar. A Thesis Submitted to Chiang Mai University and Freie University at Berlin in a Partial Fulfillment of the Requirements for the Degree of Master of Veterinary Public Health

Thrusfield, M. (2005): Sampling in: veterinary epidemiology, 3rd edition London: back well.

Wang, Ying, et al. "Polymerase chain reaction–based assays for the diagnosis of human brucellosis." Annals of clinical microbiology and antimicrobials 13.1 (2014): 1-8.

Waring R.H. and Harris R.M., 2005. Endocrine disrupters: a human risk?. Molecular and cellular

endocrinology, 244(1-2): 2-9.

Wernery U., 2014 Camelid brucellosis: a review Aetiology Impact on human health Incidence of camelid brucellosis. Rev. Sci. Tech, 33: 839-857.

Yaqoob M., Nawaz H., 2007. Potential of Pakistani camel for dairy and other uses. Anim. Sci. J., 78: 467- 475.

Yohannes, M., Gill, J. P. S., Ghatak, S., Singh, D. K. and Tolosa T. (2012): Comparative evaluation of the Rose Bengal plate test, standard tube agglutination test and complement fixation test for the diagnosis of human Brucellosis. Rev. sci. tech. Off. int. Epiz., 31(3): 979-984

Zewdie Teka and Gezahegne Mamo, 2018. Review on Epidemiology of Camel and Human Brucellosis in East Africa, IGAD Member Countries. Sci. J. Clin. Med. 6: 109.

## Annex 1. Questionnaire format

Date…….. Code no. ……

Farm structure……1…….2… 3

Farm owner……..

Occupation……

Educational status of the farm owner……

Address…….

Location …….. ……

Village…… Farm size……...

Grazing land size ……. Crop land size……..

1. How did you start camel dairy business?
   a. Inherited the enterprise
   b. Bought the enterprise
   c. Bought dairy animals
   d. Other.....

2. How did you acquire skills to raise dairy camel/farming?
   a. Agricultural training (level) ……
   b. From extension agents……
   c. From parents……..
   d. Others…….

3. What are the most common disease affecting your camel, in order of priority
   a.……
   b.……..
   c.……..
   d.……..
   e.…….
   f. ……..

5. Was there any occurrence of abortion in your farm? Yes ☐ No ☐

If your answer is yes, in which of the camel and at which time of pregnancy did it occur?

| Camel identification | Time of abortion |
|---|---|
| | |
| | |

6. What was the fate of the aborted camel (S)?

| Camel identification | Fate |
|---|---|
| | |
| | |

10: Serum sample collection format for individual camels

Region …… District ……Village……Date……

| Code No. | Sex | Age | Herd size | Breeding female history in the herd | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | Calving | Abortion | Stillbirth | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

10. What is the purpose of camel production?

a. High milk production
b. Drought mitigation
c. Bush encroachment control
d. Herd accumulation

12. Rank the use of camels:

a. Milk production
b. Transportations
c. Draught power
d. Cash income by sale

14. Cash income by selling?

a. 100%
b. 75%
c. 50%
d. 25%

16. How do you consume camel meat?

a. Cooked
b. Raw
c. Other treatment

17. Water points in different seasons

a) River Ponds
b) Traditional wells
c) others

21. What is the source of bull?

a. From own herd
b. village bull
c. Others……..

22. How do you herd Camels?

a. Separately
b. with village herds
c. with cattle
d. with small ruminants

## Annex2. Questionnaire format

Format to investigate occurrence of brucellosis in man

1. How frequent do you drink milk/ its products?

Never ☐ Rarely ☐ Frequently ☐

2.In what form (raw……. boiled or processed ) do you drink milk?

3.Has any member of the family, milkers, and other workers visited a health institution in the last six months? Y/N If yes, for what was the health problem?

4.Has any member of the family/ milker/ worker show symptoms of prolonged fever since starting being involved in dairy camel management? For how long? …….

Yes ☐ No ☐

5.Has any member of the family/ milker/ worker show the following symptoms since starting being involved in dairy camel management?

| Symptoms | Yes | No |
|---|---|---|
| Headache | | |
| Insomnia | | |
| Pain over the spine | | |
| Vague generalized pain/ aches | | |
| Pain over the joint | | |
| Pain over testes | | |
| Nervous disorders | | |

## Annex 3: farm visiting and laboratory images

**Camel from Beder farm**

**Fugure 1:** Laboratory activity (handling of RBPT technique), +ve serum for brucella infection, Plate holding with serum and stained Rosbengal Brucella Antigen with side by side and camel herds in th Field. (From top left to bottom Right.

# BIOGRAPHICAL SKETCH

The author was born in Hargeisa, capital city of Somaliland, in February 1993. He attended his Elementary in Qudhac-dheer Elementary school from 2002 to 2010 and Secondary School from 2010 to 2014 in Mahamud Ahmed Ali secondary school. After he had completed his secondary education, he took the Somaliland Schools Leaving Certificate Examination (SSLCE) and he joined University of Golis especially college of Agriculture and veterinary medicine and attended his education for four years and graduated with Bachelor of Science Degree in veterinary medicine in July 2018. After graduation, he became lecturer assistant in University of Golis specially faculty of veterinary for two months and worked until he joined the Postgraduate Programs at Bahir dar University, Ethiopia. In October 2018 he joined the Department of veterinary medicine at College of Agriculture and Environmental Sciences, Bahir Dar University to pursue his Master of Science (MSc) Degree in veterinary public health.